FRONTISPIECE

PATIENT AGE 8 RECEIVING BONE MARROW
TRANSPLANT IN A VICKERS TREXLER ISOLATOR

# BONE MARROW TRANSPLANTATION

# IN

# INFANCY AND CHILDHOOD

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# BONE MARROW TRANSPLANTATION IN INFANCY AND CHILDHOOD

## C O N T E N T S

## DECLARATION

I declare that this thesis was composed and written entirely by myself, and has not been submitted in candidature for any other degree, diploma or professional qualification.

I further declare that although the work reported in this thesis forms part of the experience of the bone marrow transplant teams in two hospitals, I have made a substantial contribution to this experience. At Westminster Children's Hospital, I was responsible for the day to day care of the transplanted children and the commissioning and organisation of all aspects of the Vickers-Trexler isolators. At the Royal Marsden Hospital, I was a paediatric member of the team caring for patients undergoing allografting and also worked in the microbiology department at the laboratory/clinician interface with special responsibility for trials of antimicrobial prophylaxis.

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# ABSTRACT OF THESIS (Regulation 6.9)

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*tle of Thesis* BONE MARROW TRANSPLANTATION IN INFANCY AND CHILDHOOD


During a bone marrow transplant there is a major risk of infection either due to the specific disease or as a result of the required preconditioning. Severely neutropenic patients benefit from receiving prophylactic antimicrobial agents within a protected environment and the thesis opens with a discussion of and a comparison between the different ways of achieving this protection and an assessment of the contribution of each component.

In the second part there is description and discussion of the practical aspects of establishing and managing isolator tents for infants and children undergoing bone marrow transplantation in the middle of an open ward in a Children's Hospital built 70 years ago. The isolators were found to be effective, practical and highly acceptable to almost all children, their relatives and the attending staff. A pathogen-free food service complementary to the degree of isolation was established and is described. Three different regimens of prophylactic antimicrobial agents were evaluated in the patients undergoing marrow transplantation and the newer regimen using co-trimoxazole found to be superior in preventing infection but to have several disadvantages. Extensive surface and orifice decontamination measures were not found to be worthwhile.

The third section discusses the practical aspects of caring for infants and children undergoing bone marrow transplantation. The management and progress of 10 infants with severe combined immune deficiency, and 10 children with severe aplastic anaemia is described in detail with particular emphasis on the immune reconstitution of the survivors. The outcome of eleven children with acute myeloid leukaemia who underwent allogeneic transplantation is described and compared with that of children who did not receive a marrow graft. Measures used to attempt germfree deliveries without caesarian section are described, evaluated and found wanting.

Viral infections after grafting remain a problem and experience with cytomegalovirus and papovavirus is discussed. Infections with herpes simplex and varicella-zoster virus after grafting are discussed with particular reference to 19 marrow transplant recipients treated with acycloguanosine which was probably effective though only in the short term.

The thesis concludes with a discussion of the requirement for marrow grafting facilities in the United Kingdom and finds that six separate centres, each with five designated beds will be the minimum required for matched sibling allografts given the present indications and this service will cost not less than £1.6 million per annum.


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BONE MARROW TRANSPLANTATION IN INFANCY AND CHILDHOOD

Detailed contents of Text

# BONE MARROW TRANSPLANTATION IN INFANCY AND CHILDHOOD

## Introduction

The first reported marrow transplant was in 1939 (Osgood et al 1939) and by 1970 (Bortin 1970) at least 203 human allografts had been reported with proven evidence of allogeneic engraftment in eleven of whom three were surviving. The early sixties had been a time of great activity in marrow transplantation with more than 133 transplants reported between 1960 and 1963. The degree of success achieved was reflected by the waning of this early enthusiasm to the extent that only twelve grafts were reported between 1966 and 1968. Almost all the reported successes were patients who had received syngeneic marrow on account of acquired aplastic anaemia.

It had been clearly appreciated by the early workers that transplants could be rejected and also that the new bone marrow could attack the recipient giving rise to fatal graft-versus-host disease (GVHD). A further problem lay in supporting the patient and keeping infection under control until competent engraftment occurred. Such patients as survived the transplant procedure frequently died due to the return of their original malignant disease because methods of eradicating this before transplantation were ineffective.

Bortin (1970) was uncertain when he reviewed the reports of 203 bone marrow transplants whether the annual number of transplants reported would dwindle to zero or climb rapidly in the next few years. However successful transplantation in severe combined immune deficiency reported by de Konig et al (1969) and Meuwissen et al (1969) gave great encouragement to those who believed that bone marrow transplantation could be effective. Now ten years after Bortin's review, over 2000 bone marrow allografts have been reported. Although definition of cure is clear only in the non-malignant disorders, the patient receiving a matched sibling allograft has an overall chance of 30-65% of being cured and in certain circumstances 85% of patients may expect to be cured.

No single advance led to this increase in the number of successful bone marrow grafts. Through the years donor selection has become more sophisticated but even so, about 20%

of patients died of acute GVHD until recently. Improved preconditioning regimens have reduced both the incidence of graft rejection in patients with severe aplasia and the incidence of recurrent disease in the patients transplanted on account of acute leukaemia. Pari passu with these important developments, great strides have been made in the ability to support patients with specific blood products until engraftment occurs. Isolation, prophylactic antimicrobial agents and systemic antibiotics have helped to prevent and treat infections during this same critical period before engraftment. In particular, confidence in the techniques grew and increasing numbers of successes have led to patients in better initial condition being subjected to grafting and this in turn has both improved the overall results and substantially reduced the morbidity of the procedure.

One centre, Seattle, has performed over 700 marrow grafts to date and by mid-1980, the International Bone Marrow Transplant Registry at the National Institutes of Health, Bethesda, had received notification from an additional sixty-four transplant teams of 251 patients who received allogeneic transplants for acute leukaemia, 275 patients who received a transplant because of severe aplastic anaemia, and 141 children who were transplanted on account of immune deficiency. (IBMTR Newsletter 9/80).

In Europe at least 160 patients with severe aplasia, including 48 children, have received marrow transplants (Gluckmann et al 1980) and 104 children are known to have received allogeneic bone marrow to treat immune deficiency (EBMT 1980).

In the United Kingdom about 250 allogeneic marrow grafts have been carried out, 102 at the Royal Marsden Hospital and about 45 each at the Westminster Hospitals and the Hammersmith Hospital. The Royal Free Hospital has performed 18 such transplants and a number of other hospitals around the country have each performed less than five. About ninety children have received allogeneic marrow in the United Kingdom, 23 at the Royal Marsden Hospital, 36 at Westminster Children's Hospital and 17 at the Hammersmith Hospital. Although this total is spread over nine years, the incidence of childhood marrow

grafts has risen rapidly with at least 40 having been performed in the past two years. I have now been fully involved throughout the whole transplantation procedure in 34 infants and children.

The increasing success of marrow grafting in childhood disease is shown in the results. Using a fully compatible allogeneic sibling donor, cure rates in combined immune deficiency now approach 60% and 35-80% of children transplanted on account of severe aplasia should be cured depending on how many transfusions they have received and whether or not they are infected. Seventy per cent of children with acute leukaemia grafted in first remission if a myeloid leukaemia and second or subsequent remission if a lymphoblastic leukaemia remain completely well and disease free a mean of fifteen months after grafting, but whether or not this represents long term cure is at present uncertain. What has become increasingly apparent over the past two years is that children accept the whole transplant procedure much better than adults as far as morbidity and survival are concerned.

It is also now well recognised that a marrow transplant can supply more than just circulating red cells, white cells and platelets. The graft can replace all cell lines derived from haemopoietic stem cells and thus monocytes, macrophages, Kuppfer cells and osteoclasts became those of the donor. Consequently the range of conditions theoretically treatable by marrow grafting includes not only 'diseases of the blood' but also some of the inborn errors of metabolism. Engraftment of cell lines bearing competent enzymes has already been shown to correct the abnormal metabolic climate resulting from certain enzyme deficiencies. These and similar diseases resulting from single gene defects are the new fields where marrow grafting must be attempted, at least until better treatments are developed.

Certain problems remain common to all bone marrow transplants whether allogeneic or syngencic. One of these is infection, most likely to be a problem during the neutropenia before engraftment. Whilst isolation techniques reduce the likelihood of the child acquiring exogenous infection during this period of neutropenia, they will not prevent infection arising from an endogenous source.

Prophylactic antimicrobial agents may reduce this danger. In addition there is some evidence of the role of micro-organisms in triggering GVHD. Prevention of both clinical infection and the acquisition of new organisms is therefore important in children undergoing marrow grafting and both protective isolation and antimicrobial decontamination play a significant part in this.

# ISOLATION AND DECONTAMINATION FOR SEVERELY NEUTROPENIC PATIENTS

## Introduction

Infection is the most common cause of death in patients with haematological malignancy or severe immunodeficiency. Infection contributed to 67% of deaths from acute leukaemia at the National Cancer Institute between 1954 and 1963 (Hersh et al 1965) and caused death in 69% of leukaemia patients between 1965 and 1971. (Levine et al 1974). Although the majority of those patients had terminal leukaemia, 25% of patients died of infection before anti-leukaemic chemotherapy could have been expected to induce remission.

A major predisposing factor to infection is prolonged granulocytopenia (Hersh et al 1965; Bodey et al 1966). Patients with aplastic anaemia, whether transplanted or not, and those leukaemia patients who fail to engraft have prolonged granulocytopenia. Although granulocytopenia is not a feature of severe combined immune deficiency (SCID) the nature of the disease and the much longer time required for competent engraftment makes these patients markedly prone to infection. 80% of infants with SCID reported to the International Bone Marrow Transplant Registry were infected at the time of transplantation and half were infected with more than one organism. (Bortin and Rimm 1977). Most of those who were uninfected had been born in a sterile manner and maintained in isolation. 85% of infants with SCID who died after transplantation died of infection. Nine of the first 100 leukaemia patients transplanted in Seattle died of infection before graft-take. (Thomas et al 1977). Infection was the most common mode of death following transplantation for aplastic anaemia (Storb et al 1978). In addition, graft-versus-host disease (GVHD) which affects a considerable number of transplant recipients delays immune recovery (Noel et al 1978) and predisposes to infection.

Since aplastic anaemia and SCID are uncommon diseases, and marrow grafting even less common, data derived during remission induction of acute non-lymphoblastic leukaemia (ANLL) must be used to consider ways of preventing infection in the

patient with prolonged neutropenia.

Schimpff et al (1972), studying 48 ANLL patients over more than two years found that half the microbiologically documented infections were caused by organisms acquired in hospital. 18% of patients harboured pseudomonas aeruginosa on admission, but a further 48% became colonised in hospital. Jameson et al (1971) reported one acquisition of staph. aureus per 20 patient weeks in hospital which would suggest that at least one in three elective transplant patients would acquire this organism during the course of their admission. Shooter et al (1963) and the PHLS report of 1965 suggest an acquisition rate double that of Jameson et al (1971). A protective environment should prevent these acquisitions.

If half the documented infections arise from organisms acquired in hospital then half arise from organisms harboured by the patient at admission (Schimpff et al 1971). No protected environment will prevent these but some form of decontamination may be effective. Removal of recently acquired microorganisms from the skin is relatively easy whereas decontamination of mucosal surfaces is much more difficult. Organisms harboured in the nasopharynx, mouth or bowel are likely to be implicated in infective processes as mucosal breaks in these sites commonly occur as sequaelae of chemotherapy or neutropenia. A number of different prophylactic antimicrobial regimens have been used often in conjunction with procedures to decontaminate the skin and mucosal surfaces.

Isolation and decontamination compared with ward care

There are numerous trials involving neutropenic patients and different combinations of a protected environment and prophylactic antimicrobial agents. Many have only small numbers, are poorly controlled, involve historical controls or are not randomised. There are no reports of a protected environment being microbiologically harmful except for the Baltimore experience of aspergillus in the asbestos fire proofing (Young and Moody 1977). Prophylactic antibiotics for decontamination may have some disadvantages.

Six randomised prospective controlled trials involving a total of 441 patients have compared ward care with care in a

protected environment (PE) plus prophylactic antimicrobial agents (PA). Five involve predominantly ANLL patients undergoing remission induction and one concerns bone marrow transplant patients. Only one of the remission induction reports (Dietrich et al 1977) includes children who form 18% of their 93 patients.

All patients in the series reported by Yates and Holland (1973) had active ANLL and half had previously received unsuccessful chemotherapy. They reported the lowest remission rate (30%) and therefore their patients were at greatest risk of infection due to prolonged neutropenia. One quarter were infected on admission, whereas Levine et al (1973) and Sleijfer et al (1980) had an incidence of infection of 12% at randomisation, while infection at that time led to exclusion from the series of Schimpff et al (1975). In the multicentre study of Dietrich et al (1977), 45% of patients were infected at randomisation, 30% of whom were dead by 50 days, compared with 15% of those who were not infected at randomisation. Buckner et al (1978) reported on marrow graft recipients, one quarter of whom were infected at randomisation.

Results

Table 1 shows some results from these trials.

The two remission induction series with 25% or more patients infected at randomisation show least benefit in PEPA. The studies by Levine et al (1973), Schimpff et al (1975) and Sleijfer et al (1980) where infection at randomisation was either 12% or absent show benefit from PEPA with a marked reduction in the number of infections and bacteraemias per patient and in the number of deaths from infection compared with the patients on the open ward (Table 1). Schimpff et al (1975) also found those undergoing PEPA to have a higher remission rate, a finding not generally confirmed.

The period of severe neutropenia is usually shorter in transplant patients than in those undergoing remission induction. Buckner et al (1978) reported a randomised trial of PEPA from the Seattle transplant unit. Their patients experiencing PEPA had one-third the incidence of infection and half the incidence of bacteraemia compared to their control group. 11% of the PEPA

Table 1

INFECTIONS, BACTERAEMIAS AND DEATHS FROM INFECTION WITH AND WITHOUT PROTECTED CARE (PEPA)

| Author | patient numbers | level of care | proven infections per patient | bacteraemias per patient | deaths from infection |
|---|---|---|---|---|---|
| Yates and Holland 1973 | 31 | - | .83 | | 6 (19%) |
| | 20 | PEPA | .25 | | 2 (10%) |
| Levine et al 1973 | 28 | - | .53* | .25 | 6 (21%) |
| | 22 | PEPA | .27* | .09 | 0 (0%) |
| Schimpff et al 1975 | 21 | - | 3 | .76 | 11 (52%) |
| | 24 | PEPA | 1.13 | .17 | 4 (17%) |
| Dietrich et al 1977 | 51 | - | 1.06 | | 8 (16%) |
| | 42 | PEPA | .83 | | 4 (10%) |
| Sleijfer et al 1980 | 55 | - | .69 | .14 | 9 (16%) |
| | 58 | PEPA | .16 | .05 | 0 (0%) |
| Buckner[1] et al 1978 | 44 | mask isol. | 1.39 | .52 | 2 (4%) |
| | 45 | PEPA | .44 | .22 | 1 (2%) |

* severe infections only

1 excludes interstitial pneumonia

PEPA (protected environment, prophylactic antibiotics)

patients required therapeutic granulocytes compared to 50% of the unprotected patients. However, there was no difference between the groups in the number of deaths from infection, nor any improvement in the overall results of transplantation.

These data show that infections are less common, less often fatal and the measures required to treat infection are less complicated when patients are nursed in a protected environment with prophylactic antimicrobial agents. This is particularly true for patients who are uninfected at the time of commencing PEPA and although the benefit may be less marked for patients undergoing bone marrow transplantation there is still a benefit not only for the patients but also for the staff. Other data suggest that graft-versus-host disease (GVHD) is less common in the decontaminated patient (Vossen 1980). For these reasons the combination of isolation and decontamination must be recommended for neutropenic patients, and although the final benefits are less well documented such a policy seems wise for patients undergoing bone marrow transplantation. However, it is worth examining the two components, isolation and antimicrobial decontamination, separately to see what each contributes to the protection of the patient.

## THE PROTECTED ENVIRONMENT

The use of a protected environment in bone marrow transplantation was regarded as speculative in 1975 (Thomas et al 1975) and there is still no proof that isolation and decontamination improves the overall survival of these patients but benefits referred to above have been clearly demonstrated (Buckner et al 1978). The aim of a protected environment is to prevent the child acquiring exogenous organisms from either the environment or their attendants. The basic requirements of a protected environment are that the physical structure be initially gnotobiotic and can be easily so maintained; suitably treated supplies can be introduced in an aseptic manner; adequate patient care is feasible and the environment acceptable to both the patients and attending staff who must not require excessive training. In addition, the protected environment must be able to function within the limitations of the staff available and the hospital in which it is being used.

Different methods of achieving such an environment are available and each has advantages and disadvantages but each system is only as good as its weakest aspect.

Sources of exogenous infection

Although sources of exogenous infection for the hospitalised child include such communal ward equipment as baths (Alder and Clee 1966) and toilets (Newsom 1972), stethoscopes (Gerken et al 1972), respiratory apparatus (Lowbury et al 1970), ward food (Shooter et al 1971), drugs and disinfectant solutions (Ayliffe et al 1969), without doubt the major sources of cross-infection lie in the attending staff, other patients and to a lesser extent, visitors. A quarter of the normal population carry pseudomonas spp., 5-20% carry strep. pyogenes and up to 40% carry staph. aureus (Bagshawe et al 1978). The hands of attendants are frequently contaminated in intensive care units (Lowbury et al 1970) and one-third of hospital staff may carry staph. aureus or gram negative bacilli on their hands (Schimpff et al 1972). In Birmingham, 12% of nurses carried staph. aureus on their fingers (Ayliffe et al 1979) and the spread of this organism is particularly likely if the carrier is also a skin disperser (Hare and Thomas 1956), a situation more common in males (Bethune et al 1965). Nurses uniforms are frequently contaminated (Speers 1969) as is bedding with which they have just dealt (Lidwell et al 1974). Common respiratory tract pathogens are also readily transferred from staff to patients (Hall et al 1976).

Staff procedures to prevent cross-infection

Methods to prevent cross-infection from the staff are required in all protected environments where there is direct physical contact between the patient and the attendants. The most important is hand-washing which will quickly remove recently acquired organisms but for this to be performed properly is unusual (Bagshawe et al 1978). Hibiscrub (4% chlorhexidine in detergent) is a useful skin antiseptic (Lowbury and Lilly 1973) which leaves some residual antiseptic activity on the hands but the ward containers may become a reservoir for pseudomonads if not frequently replaced. Hibisol (0.5% chlorhexidine in isopropyl

alcohol) is also effective and may have a less drying effect on the hands. Iodinated compounds, e.g. Betadine are also anti-viral in action. The skin of some people is sensitive to these preparations so a choice may be necessary.

Protective clothing is required to prevent contact transmission. This may vary from a complete change of clothing before each entry into the protected area to putting a protective cover over the attendant's normal clothes on each occasion. Disposable plastic aprons cover only at the front but are impervious to fluids and are become much less contaminated with staph. aureus than the fabric of nurses' uniforms (Lidwell and Towers 1972). Gowns cover the shoulders and arms but do not cover below the knees which boiler suits will do. Cotton or fibre protective clothing is not normally of the close-weave pattern which will reduce staphylococcal dispersal by susceptible people. Although special non-woven material (Fabric 440, Johnson and Johnson) may be used (Hill et al 1974) a member of staff who disperses staphylococci is better excluded from the clinical team. Linen gowns are more durable than those made of woven fibre (Fabric 450, Johnson and Johnson) and are only slightly more expensive. Providing a fresh gown is used on each occasion, it is unnecessary for these to be sterile. If sterile gowns are required, those made of fibre will survive three trips through an autoclave but cannot be washed in the hospital laundry.

Protective clothing traditionally includes masks, gloves, hats and overshoes. Thin paper masks become very poor barriers after several minutes but they have a value in limiting droplet spread during speech. Industrial masks are more durable and also more comfortable. Thim polythene disposagloves rapidly disintegrate, are awkward for venepuncture and make bathing or applying lotions to a child difficult. If gloves are required for any length of time, surgical gloves are preferable. The use of gloves must not lead staff to omit hand washing and there is no evidence that gloves are superior to properly washed hands in preventing cross-infection. Paper hats are of limited effectiveness but since hair is so readily colonised it is reasonable to cover all hair including beards (Lidwell 1977).

Disposable plastic overshoes have a value only in promoting general cleanliness.

Washing the hands and putting on a gown and gloves takes 1 minute 30 seconds (Tyrrell et al 1977). If a cubicle is entered three times an hour, 7.5% of a nurse's time will be taken up with these procedures. To wash the hands and forearms for 5 minutes is impractical. If the materials used are disposable, the material cost of each entry into the cubicle will be about 75 pence. Compliance with these procedures is a further problem. Tyrrell et al (1977) reported from their purpose-built isolation unit that half the hand washes before donning protective clothing were omitted which is unsatisfactory as hand contact is so effective in cross-infection.

The structure of the isolation facility

The physical structure of the isolation facility is important. Surfaces should be smooth, impervious and easily cleaned. "Self-sterilising" surfaces are not effective. Toilets should not create aerosols on flushing especially if positive pressure air is being used as these aerosols will be dispersed downwind to non-isolated patients. Taps on the sinks should be elbow operated and the stream of water not cause spray by directly hitting the plug-hole. Sinks must be deep enough to allow washing to the elbows without splashing nearby walls which will cause plaster and grouting to be continually damp. Sinks should not have an overflow, a site which encourages the growth of pseudomonads although the significance of this site has been debated. (Hughes et al 1978). Water traps in the sink bend are also a site for pseudomonads but the absence of a trap may lead to odours arising from the drains. Electric boiling systems for water traps have not been generally adopted.

Doors leading into the protected area must fit well. Swinging doors take up space but sliding doors are less air tight and the runners are difficult to keep clean. While swinging doors may be pushed open, sliding doors will contaminate clean hands. Mechanical or photoelectric methods of opening are possible, but doors should close automatically.

Suitable supplies should enter the protected area without

a large door having to be opened each time. In isolation systems dependent on air curtains to maintain their integrity, this is not a problem but in solid structures, e.g. cubicles, through-hatches with safety locks to prevent both hatch doors being open at once are frequently used. They are more effective in preventing air-borne contamination if part of an independent negative pressure gradient draining elsewhere (Bagshawe et al 1978) but this becomes both expensive and complicated. Ultra-violet light in a closed hatch will reduce air-borne contamination, but areas in shadow will not be sterilised and the effects are greatly reduced by humidity.

Positive pressure filtered air

These systems have the benefit of increasing the control over the patient's environment and when available in each isolated area, airborne cross-infection between isolated patients will not occur. Positive pressure air allows air conditioning and has a dilutional effect on such airborne organisms as are in the patient's environment. Pressure differences at different sites within and without the protected environment will allow air flow to be in the required direction. While 5$\mu$ filters may be adequate, HEPA filters (0.3$\mu$) are more effective. They trap bacteria and viruses adherent to dust particles. Individual cubicle terminal air filtration is preferable but more expensive than a single filter bank. Easy maintenance is of prime importance, and as much servicing access as possible should be outside the protected area. Diffusers fitted where positive pressure air enters the cubicle are necessary to avoid local turbulence and draughts. They collect dust and debris so should be easily cleaned. Special designs have reduced flow noise considerably.

There are however other considerations with positive pressure filtered air. A single air-conditioning source serving a number of cubicles gives particularly fickle variations in pressure and temperature within different cubicles (Hambreus et al 1972). Contaminated air passing from a cubicle to a common corridor may constitute a hazard to non-isolated patients (McKendrick and Emond 1976), but arranging for each cubicle to have an extraction system that balances the air input is technically complicated.

An open door will double the air flow required to maintain the slight positive pressure existing in a room which has seven air changes per hour, but the provision of an anteroom "air-lock" will maintain the differential providing the outer door remains closed. Because of convection currents a difference of $1C^{o}$ across an open door will by itself double the flow required to maintain a positive pressure. Few air flow systems have built-in compensators for these circumstances.

Methods of isolation

Mask isolation with hand washing is the simplest form of protective isolation. All methods except laminar air flow require visible barriers and due psychological note must be taken of this. Patients feel a sense of abandonment if totally isolated, especially if all visitors are excluded and their disease serious. Wherever possible, certain visitors should be allowed and there is little justification for totally isolating the child from both parents.

Cubicles

Unless purpose-built, hospital cubicles have fittings making them difficult to keep clean although initial substantial sterility may be possible using formaldehyde vapour. They rarely have an anteroom in which staff may wash and gown-up. Integral toilets are unusual and the plumbing is difficult to keep clean. Glass windows make for poor sound transmission and taking x-rays usually involves breaking isolation. Filtered air would be difficult to install and there is rarely space for the power plant required. It is difficult to derive a properly protected environment from a normal hospital cubicle but considerable protection can be derived by careful hand washing, protective clothing and the use of a cooked food diet.

Laminar Air Flow Units

Laminar air flow (LAF) units with HEPA filters are commonly used to provide a protected environment. The theory is to provide an undirectional non-turbulent flow of sterile air. The units are mobile and can be used in a cubicle or an open ward or else a cubicle can be constructed with one whole wall as the

LAF source - a so called LAF room. Some systems have two feet of curtaining around the edge of the LAF source and depending on the size of the unit a variable number of beds may be placed in the sterile air flow.

Designs include vertical downflow to a whole floor extract, horizontal flow to either the open ward, a single floor extract or a full wall extract.

Laminar air flow is achieved with all these designs except the one with a single floor extract where the direction of flow varies. However when furniture, including the bed, or attendants are in the air flow, the laminar nature is lost because of downstream turbulence and the convection effects of human body heat. If an attendant is 'upwind' of the patient, the efficacy of the unit is lost as when the power fails or the filters clog. LAF units depend entirely on air flow for their integrity.

The usual horizontal air velocity is 30-50 feet/minute but this may be increased to 90 feet/min. The volume of air required for a single bed unit (about 2000 cubic feet/minute) gives a considerable diluting effect on any airborne organisms. A two bed unit requires a flow of 35 feet/min to prevent contamination between beds six feet apart (Lidwell and Towers 1969). Laminar downflow requires about two-thirds the horizontal flow rates for satisfactory operation.

With no physical barrier there is great ease of access and communication. This ease of access is almost a disadvantage and requires discipline as the invisible curtain of air must not be breached. Active children, careless visitors and insects can easily penetrate the barrier. Full protective clothing must be worn when entering the protected area. To avoid the necessity for this, one design incorporates a moveable wall with gloves and sleeves (Penland and Perry 1970). Certain procedures can be performed via these sleeves avoiding the necessity of entering the protected area.

PVC isolator tents

Plastic iolators with positive pressure HEPA filtered air have been used for animals since 1957 and for humans since 1964. (Schwartz and Perry 1966). These early models "Life-Islands"

were made of heavy PVC which had poor translucency. They had heavy insensitive gloves, poor access and viewing of the patient and with the early designs staff had to wear protective clothing when caring for the patients. Isolators completely enclosing the patient, the bed and the furniture and requiring the attending staff to use whole body PVC suits attached to the walls of the isolator were not greeted with enthusiasm by Robertson et al (1968) who in particular felt that children aged nine or less would prove too untidy and messy for nursing in isolators. Those designs and the paediatric isolator reported by Barnes et al (1968) had complicated hatches for entering sterile supplies. The isolators developed in 1972 (Trexler et al 1975) overcame a number of the difficulties. The shape was altered to give better visibility and the bed and furniture left outside the isolator but projecting into it via reflections of PVC, so therefore usable by the patient. The gloves and PVC were made less heavy and the supply entry system made very simple. Other designs (Dietrich et al 1975) have included a concertina system of the walls so to allow greater space for the patient therein confined.

The protected area is essentially a closed PVC bag which is only opened to introduce supplies. Apart from the sterilisation of these, no effort is required to maintain the integrity of the protected environment. The system depends on HEPA filtered positive pressure air with a physical barrier which also gives protection in the event of a power failure. Small leaks or puncture holes do not result in loss of protection partly because of the pressure differential but also because the electrostatic charge developed when air passes through a small hole causes particles to adhere to the PVC. Further details of the design and use of isolators follow page 50.

Microbiological surveillance of the isolation system

An appropriate programme is necessary for any isolation system, but must be chosen to give the required information. Initial cultures will show the sterility of the system prior to entry of the patient but the intention of isolation is to prevent exogenous contamination of the patient with micro-organisms from the staff or environment.

Routine cultures from various sites on the patient with antibiotic sensitivity testing of any likely pathogens occasionally modifies the immediate treatment if presumed infection occurs. This is particularly true of a patient with previous hospital admissions who may harbour antibiotic resistant organisms. A more immediate use of routine patient cultures is that phage typing of staphylococci and pseudomonads and biotyping of enterobacteriaceae will identify cross-colonisation and a breakdown in the protected environment.

Patient screening is more effective than the routine monitoring of the flora on the attendants and does not raise the difficult questions of how often should staff be studied and what should be done with the results. Since 30% of staff carry nasal staph. aureus intermittently and 25% have pseudomonads in the stool are these staff to be transferred to another ward? Admittedly patient monitoring only identifies post-hoc cross-infection but these microbiological studies are only a way of documenting that the staff are maintaining the standards to which they were trained. This need for training applies equally to all staff, medical, nursing and ancillary, who are in contact with the patients. Should cross-infection occur, then a specific carrier search can be undertaken.

Environmental monitoring using settle plates or air sampling gives some indication of airborne organisms. Settle plates are cheap, easy to use and their presence is psychologically useful. By the duration of exposure, settle plates record both busy and quiet periods of time and can be placed strategically throughout the protected environment. Culturing from surfaces is of little value as these are usually kept clean. Accumulations of dust are microbiologically hazardous but their very presence indicates a breakdown in house-keeping which needs to be remedied.

The value of protective measures

Each aspect of a protected environment contributes some protection but in the absence of certain basic measures, sophisticated techniques add nothing. Lidwell and Towers (1972) used a LAF unit for patients recovering from thoracic surgery. Normal nursing techniques were used and the acquisition of

of staphylococci and gram negative bacilli compared between this unit and their ordinary ward. Twenty per cent of the ward beds became contaminated with staphylococci compared to 10% of those in LAF. There were 13 acquisitions of staphylococci in the ward patients and 9.3 in the LAF patients per 100 patient days and 8 acquisitions of gram negative bacilli in ward patients compared to 7.5 by the LAF patients in a similar period of time. Thus the protective effect of sterile air without any change in nursing techniques is minimal.

A simple physical barrier (a hospital cubicle) will reduce the acquisition of staphylococci (Parker et al 1965) but only if the doors remain closed. The imposition of a physical barrier was effective in the burns unit reported by Lowbury et al (1971) who found that 65% of patients in the open ward acquired pseudomonads compared to 10% of those who were in an isolator. This difference was maintained whether or not the isolator had filters attached, i.e. the use of sterile air gave no additional benefit in excluding pseudomonads. They also reported that the isolator protected to a degree against the acquisition of multiple antibiotic resistant staphylococci but did not affect the acquisition of proteus spp. and some other coliform bacilli. They were not using a sterile diet and it is likely that this contributed to the new acquisitions.

The use of a specially prepared diet is important as shown when Vossen et al (1972) compared their ultra-clean rooms (HEPA filtered) with their hospital cubicles. Food and drug supplies were not decontaminated but full protective clothing was used in both systems. No cultures from the cubicles were sterile and 40% of surface cultures in the ultra-clean rooms grew organisms. Eight per cent of food samples contained very high bacterial counts. The protective effect of the HEPA filtered air in maintaining the cleanliness of the rooms was rapidly lost owing to these contaminated supplies.

It is therefore difficult to separate out the benefits contributed by each part of a protected environment and it is impossible to separate the effects of a highly trained nursing team when results from an occupied protected environment are quoted. However it is clear that

a) a physical barrier protects
b) sterile air is useless in the presence of contact transmission
c) food and drug supplies unless specially prepared will negate the benefits of preventing contact and airborne transmission.

To decide the value of a protected environment by itself it is necessary to review results obtained when this was the only variable. Six randomised studies on patients with severe neutropenia are available as are several other studies relating to more general medical circumstances.

Results - colonisation

Jameson et al (1971) reported a reduction in staphylococcal acquisition and many fewer patients acquiring pseudomonads in their air-filtered cubicles and isolators but found no difference between these two forms of isolation. (Table 2 ). Dietrich et al (1977) reported a multicentre trial with the protected group receiving sterile food and supplies. These patients had proven contamination and colonisation reduced by a third. Schimpff et al (1975; 1978) moved from a normally ventilated hospital to one where the hospital air conditioning was HEPA filtered without recirculation. The reduction in colonisation by potential pathogens was similar in both their additionally protected environments compared with the open ward but the difference resulting from the HEPA filtration of the ordinary air in their new hospital was convincing in its own right and further HEPA filtration did not significantly reduce the rate of colonisation. The Hammersmith group (Trexler et al 1976) knew of no organism gaining entrance to their isolators over 770 days, and the Westminster group over 242 days (Watson et al 1977) also identified no exogenous organisms in their isolators.

- acquired infection

Evidence for the beneficial effects of a protected environment (PE) on the incidence of or death from infection is clear in only some studies and particularly those in which prophylactic antimicrobial agents were also given and there was a low incidence of infection at entry to the study (Table 3). The studies of Levine et al (1973) and Schimpff et al (1975 and 1978) show considerable benefit but that of Klastersky et

Table 2

BACTERIAL ACQUISITION IN DIFFERENT ENVIRONMENTS

| Environment (ref 1) | | Per 100 patient weeks | |
|---|---|---|---|
| | | Acquisitions of staphylococci | patients acquiring pseudomonas |
| Open ward | | 5.0 | 21% |
| protected environment | cubicles | 1.3 | 8% |
| | isolators | 1.4 | |

| Environment | contaminations per patient per week (ref 2) | colonisations per patient per week | | |
|---|---|---|---|---|
| | | ref 2 | ref 3 | ref 4 |
| Open ward | 2.35 | 0.6 | 0.58 | 0.15 |
| Protected environment (isolators or LAF) | 1.59 | 0.41 | 0.42 | 0.1 |


1 Jameson et al 1971

2 Dietrich et al 1977

3 Schimpff et al 1975

4 Schimpff et al 1978

Table 3

COMPARISON OF SEVERE INFECTIONS AND DEATHS FROM INFECTION WITH OR WITHOUT A PROTECTED ENVIRONMENT

| Author | Total no of patients | Nature of Protected Environment (PE) | Severe infections/ patient | | Deaths from infection | |
|---|---|---|---|---|---|---|
| | | | In PE | No PE | in PE | No PE |
| Yates & Holland 1973 [1] | 87 | LAF and isolators | .54 | .63 | 5 (14%) | 7 (13%) |
| Levine et al 1973 [2] | 66 | LAF and isolators | .23 | .58 | 0 (0%) | 9 (23%) |
| Klastersky et al 1974 [3] | 30 | Isolators | .63 | .5 | 6 (38%) | 5 (36%) |
| Schimpff et al 1975 [2] | 43 | LAF rooms | .33 | .74 | 3 (13%) | 7 (37%) |
| Dietrich et al 1977 [3] | 95 | LAF and isolators | 1.02 | 1.06 | 5 (11%) | 8 (16%) |
| Schimpff et al 1978 [2] | 22 | LAF within a HEPA environment | .27 | .33 | 1 (9%) | 3 (14%) |

1 PA variable in both arms. PE group had less severe neutropenia

2 All patients received PA. PE group had more neutropenia than control

3 All patients received PA
PE group had more neutropenia than control

PA prophylactic antimicrobial agents

al (1974) who excluded patients infected at randomisation, showed no benefit related to isolation per se in their 16 isolated patients. However most studies show that a protected environment is useful for patients who are in good condition when they commence their treatment. The study of Yates and Holland (1973) included many patients who had already received unsuccessful remission induction therapy for acute myeloid leukaemia and the patients reported by Dietrich et al (1977) had an infection incidence of 45% at randomisation.

Respiratory infections are less common in isolated patients (Yates and Holland 1973; Schimpff et al 1975; Dietrich et al 1977) as are pseudomonas infections (Yates and Holland 1973; Levine et al 1973) but LAF within a HEPA filtered hospital did not decrease respiratory infections further, although it did increase the proportion of patients who never had an infection (Schimpff et al 1978).

Schimpff et al (1975) found their isolated group took a mean of 20 days to develop their first infection compared with the control group mean of 15 days but Levine et al (1973) and Schimpff et al (1978) found no such difference. No real difference in the number of days with a fever greater than $38^{o}C$ has been reported (Priesler et al 1970; Dietrich et al 1977). Priesler et al (1970) found their isolated group spent 10% of their days on therapeutic antibiotics compared with 25% of their control group.

Summary

From these data, the balance is in favour of providing a protected environment for the severely neutropenic patient although the use of prophylactic antimicrobial agents would seem to add considerably to the benefits achieved. The type of isolation facility to be used will depend on many local factors but simple hospital cubicles have many disadvantages and therefore a specially constructed facility is preferable.

PROPHYLACTIC ANTIMICROBIAL REGIMENS

There are considerable differences in the content of the oral prophylactic antimicrobial regimens used in different centres. The use of nalidixic acid and more recently co-trimoxazole, absorbable antibacterial agents, has given fresh impetus to the concept of systemic antibacterial prophylaxis as an alternative to non-absorbable antimicrobial agents which were given solely to reduce the bacterial content of the gastro-intestinal tract. The majority of patients who have received these antimicrobial prophylactic agents are adults undergoing remission induction of acute myeloid leukaemia (AML) but data relating to bone marrow transplant recipients are slowly accumulating. Some randomised trials involving prophylactic antimicrobial agents in AML patients have had a protected environment common to both those who received and did not receive prophylactic antimicrobial agents and therefore only limited comparison between different decontamination regimens is possible.

Sterility of the stools

Before 1975 there was interest in the percentages of sterile stool cultures with papers recounting long lists of the microbes eliminated. Recently, there has been much less interest in the elimination of all micro-organisms, although this may be relevant to the prevention of graft-versus-host disease in marrow graft recipients. Stool cultures from decontaminated patients may not show organisms despite their presence on gram staining of the stool. Culture may be rewarded if the anti-microbials are eluted from the stool. When decontamination is discontinued, many organisms reappear which were present before decontamination commenced, but were suppressed for the duration. In addition, the results of culture may depend on how the specimen was obtained and transported, which culture media were used and under what conditions.

The early rotating antibiotic regimen of Schwartz and Perry (1966) became replaced in North America by gentamicin, vancomycin and nystatin (GVN), first reported by Bodey et al (1968). The efficacy of GVN was confirmed by Bodey and Rosenbaum (1974), albeit using historical controls (table 4). Priesler et al (1970) combined the results of 20 patients, some in a

Table 4

COMPARISON OF GUT ANTIMICROBIAL REGIMENS

| Author | Regimen | Patients with consistently sterile stools | Patients with elimination of pathogenic bacteria | |
|---|---|---|---|---|
| Priesler et al 1970 | Rotating | 1/11 | 4/11 | 30% |
| | GVN | 0/9 | 8/9 | 89% |
| Bodey and Rosenbaum 1974 | PPVF | 2/19 | 14/19 | 74% |
| | GVN | 12/46 | 42/46 | 91% |

Rotating regimen (Schwartz and Perry 1966)

Nystatin 2 x $10^6$ units daily
plus

Days 1-4 Bacitracin 100,000 units daily
Neomycin 3.5 gms daily
Sulfathalidine 5 gms daily

5-9 Bacitracin 100,000 units daily
Neomycin 3.5 gms daily
Polymixin B .85 gms daily

10-16 Paromomycin 1.5 gms daily
Kanamycin 3.5 gms daily

PPVF

Paromomycin 3 gms daily
Polymixin B 420 mg daily
Vancomycin 1.5 gms daily

various antifungal agents

GVN

| Priesler et al 1970 | Bodey et al 1974 |
|---|---|
| Gentamicin 800 mg daily | 1200 mg daily |
| Vancomycin 2 gms daily | 1.5 gms daily |
| Nystatin 4-12 x $10^6$ units daily | 21 x $10^6$ units |

protected environment and some receiving a pathogen-free diet which may explain why so few achieved consistently sterile stools compared with the results of Bodey and Rosenbaum (1974) (table 4 ). More patients receiving GVN had stool pathogens eliminated that those receiving rotating antibiotics or PPVF. Bodey and Rosenbaum (1974) did not report differences between their groups as regards the suppression of particular flora but fungi were often present in the stools. Bodey and Rosenbaum (1974) found nystatin in their doses to be slightly more effective than candicidin. Although amphotericin was also an option in the PPVF regimen the results are not reported.

Three other series using oral GVN in a protected environment have been reported with details of the constancy and completeness of microbial suppression in the stools. (Table 5). Schimpff et al (1975) found consistent complete suppression of all stool flora in all of 24 patients. Levi et al (1973) reported consistent bacterial suppression in 7 of 21 patients but a further 11 of the 21 had fungi consistently present in the stools. Levine et al (1973) showed no bacteria in 94% of the stools cultured from their 22 patients but make no mention of consistent complete microbial suppression in any one patient. One stool in five contained fungi on culture. Clearly the GVN regimen, which has also been given to infants (Park et al 1973) can give considerable suppression of stool bacteria but in the hands of most authors, this considerable bacterial suppression is associated with the isolation of fungi on many occasions.

Elimination of pathogens from the stools

Since 1975 interest has moved from total stool microbial suppression to interest in the elimination of pathogens. Lactobacilli, strep. faecalis, bacteroides and diphtheroids are the organisms commonly considered acceptable in the stool. It is the elimination of the enterobacteriaciae, pseudomonads and fungi that is particularly useful, and it is worth remembering that although infections with exotic organisms do occur, the vast majority of infections are due to common well-recognised pathogens.

Table 5a compares the efficacy of various antimicrobial regimens in eliminating pathogens from the stools. Although the reported results range from 0% to 100% success in elimination

Table 5a

COMPARISON OF GUT ANTIMICROBIAL REGIMENS
ANTIMICROBIAL REGIMENS QUOTING
ELIMINATION OF PATHOGENS FROM STOOLS

| AUTHOR | REGIMEN | PE | PATIENT NUMBERS | NO. WITH CONSISTENT ELIMINATION OF PATHOGENS (see text) |
|---|---|---|---|---|
| Priesler et al 1970 | Rotating | NE | 11 | 1 (9%) |
| Priesler et al 1970 | GVN | NE | 9 | 0 |
| Bodey and Rosenbaum 1974 | PPVF | + | 19 | $\geq$ 2 ($\geq$11%) |
| Levi et al 1973 | GVN | + | 21 | 7 (33%) |
| Bodey and Rosenbaum 1974 | GVN | + | 46 | $\geq$12 ($\geq$26%) |
| Schimpff et al 1975 | GVN | + | 24 | 24 (100%) |
| Buckner* et al 1978 | GVN Plus | + | 12 | 9 (75%) |
| Dietrich et al 1973 | BNNP[1] | + | 21 | 0 |
| Dankert et al 1978 | BNNP | + | 28 | 4 (14%) |
| Guiot and Van Furth 1977 | Multiple[4] | + | 9 | 2 (22%) |
| Guiot 1980 | Multiple[4] | ? | 16 | 0 (0%) |
| Storring et al 1977 | FRACON[2]+A | + | 46 | 45 (97%) |
| Watson and Jameson 1979 | NEOCON[3]+A | + | 34 | 31 (91%) |
| Sleijfer et al 1980 | Multiple[5] | NO | 58 | 16 (28%) |

* Bone marrow transplant patients

PE - protected environment

NE - not evaluable

Table 5b

[1]BNNP - Bacitracin 160,000 units/day
Neomycin 3 gms/day
Nystatin 6 x $10^6$ units per day
Polymyxin B 200 mg/day

[2]FRACON+A
Framycetin 2 gms/day
Colistin 6 x $10^6$ units/day
Nystatin 2.4 x $10^6$ units/day
Amphotericin 800 mg/day

[3]NECON+A
Neomycin 1 gm/day
Colistin 3 x $10^6$ units/day
Nystatin 1.2 x $10^6$ units/day
Amphotericin 800 mg/day

[4]
Neomycin or Kanamycin 1 gm/day
Polymyxin B or Colistin 400 mg/day
Nalidixic acid 4 gms/day
Amphotericin or Miconazole 1-2 gms/day

[5]
Choice from the following:
Nalidixic acid 8 gms/day
Co-trimoxazole (480 mg Trimethoprim/2400 mg sulphamethoxazole/day)
Polymixin E 800 mg/day
+ Amphotericin elixir 2 gms/day

(ADULT DOSES)

of pathogens some of the differences may be interpretative rather than real. Schimpff et al (1975) using twice as much vancomycin as in other regimens report excellent results but they admit to this only being when the patients tolerated the regimen. Similarly the data of Buckner et al (1978) only records the 12 patients who took and tolerated all their GVN. Only 20% of their 46 patients receiving GVN achieved consistent suppression of stool pathogens, results not dissimilar to those of Levi et al (1973) and Bodey and Rosenbaum (1974).

However, effective as the Schimpff et al (1975) version of GVN was, there is little difference between it and the FRACON or NEOCON regimens reported by Storring et al (1977) and Watson and Jameson (1979). There is little to recommend the BNNP regimens, those of Guiot and Van Furth (1977) or Guiot (1980), should the object be the consistent elimination of pathogens from the stool. The regimen of Sleijfer et al (1980) was chosen from three antimicrobial agents on the basis of an antibiogram. This regimen included co-trimoxazole and when given without a protected environment achieved consistent elimination of pathogens from the stool in 28% of patients.

Other regimens have been reported but not with stool culture results. Keating and Penington (1973) used framycetin 750 mg twice daily without isolation, and co-trimoxazole has been used as a sole agent (Gurwith et al 1978) and in combination with FRACON (Enno et al 1978). Rodriguez et al (1978) reported 145 patients, some receiving oral absorbable plus non-absorbable antimicrobial agents and some receiving similar systemic agents.

Days with fever or receiving therapeutic antibiotics

All comparisons with each control group except that of Dietrich et al (1973) showed a benefit to those receiving prophylactic antimicrobial agents. (Table 6 ). All authors defined a fever day as a day when the patient's temperature was greater than 38°C for more than either four or six hours, except Buckner et al (1978) who used 38.3°C and Dankert et al (1978) who used 39°C. The duration and degree of neutropenia where stated were broadly similar in all the reports with the exceptions of Gurwith et al (1978) whose patients each had less than eight days of severe neutropenia, Keating and

Table 6

COMPARISON OF GUT REGIMENS

days with fever and days receiving therapeutic antibiotics

| Author | Regimen | PE | Number of patients | Percent. of days with fever | Percent. of days on therapeutic antibiotics |
|---|---|---|---|---|---|
| Keating and Penington 1973 | - | - | 66 | 40% | 47% |
| | Framycetin | - | 38 | 27% | 39% |
| Dietrich et al 1973 | - | + | 9 | 18% | ND |
| | BNNP | + | 21 | 21% | ND |
| Dietrich et al 1977 | BNNP | + | 42 | 20% | ND |
| Dankert et al 1978 | - | + | 27 | 25% | ND |
| | BNNP | + | 23 | 11% | ND |
| Storring et al 1977 | - | + | 49 | 26% | 64% |
| | FRACON | + | 46 | 18% | 45% |
| Enno et al 1978 | FRACON | + | 16 | ND | 88% |
| | FRACON + TMP/SMX | + | 14 | | 31% |
| Gurwith et al 1978 | - | ? | 42 | 37% | ND |
| | TMP/SMX | ? | 43 | 17% | ND |
| Watson and Jameson 1979 | NEOCON | + | 34 | 14% | 47% |
| Rozenberg-Arska et al 1980 | - | - | 20 | 37% | 47% |
| | TMP/SMX | - | 18 | 29% | 22% |
| Buckner* et al 1978 | - | - | 45 | 39% | 66% |
| | GVN | + | 46 | 28% | 52% |
| Watson and Jameson* 1979 | NEOCON | + | 14 | 14% | 36% |

PE - protected environment ND - no data

*marrow transplant patients

Penington (1973) whose group receiving framycetin had twice as much severe neutropenia as their historical controls and Dankert et al (1978) whose treated group had half as much severe neutropenia as the control group. GVN is not represented in the non-transplant patients in table 6. However regimens using framycetin or neomycin seem to give similar results to those employing co-trimoxazole. In view of the degree of fever required by Dankert et al (1978) and the distribution of their patients' neutropenic days, only Dietrich et al (1977) indicate any real value in BNNP but without appropriate controls.

There are two marrow transplant series reported, neither of which have entirely appropriate controls. Buckner et al (1978) used GVN with an average in-patient stay of 45 days compared to Watson and Jameson (1979) who used NEOCON during an average in-patient stay of 32 days. Buckner et al used a slightly higher fever threshold yet had twice as many fever days and 50% more antibiotic days than the Royal Marsden acute leukaemia transplant series. One-third of the Seattle transplant patients suffered aplastic anaemia but these had disproportionately few infection although their number of fever days and days on antibiotics is not specially quoted.

All the regimens where each patient had more than 10 days with a polymorph count of less than $0.1 \times 10^9/l$ gave results of fever on about 20% of days and therapeutic antibiotics required on about 40% of days.

Infections per patient

Eleven reports in which prophylactic antibiotics were the only variable are summarised in Table 7. Of seven studies during remission induction of AML showing prophylactic antibiotics to be useful, four included protective isolation. In not one of the three studies showing no benefit to result from prophylactic antimicrobial agents was protective isolation used. The two best results with GVN were very similar to FRACON in a protected environment, to the remarkable complex regimens of Rodriguez et al (1978) and to the simple use of co-trimoxazole by Rosenberg-Arska et al (1980) who did not use a protected environment. Unfortunately the degree of neutropenia is not stated in that study, nor in the study by Sleijfer et al (1980).

Table 7a

## COMPARISON OF ANTIMICROBIAL REGIMENS

Infections

| Author | No. of patients | Proven infections per patient | Regimen | No. of patients | Proven infections per patient |
|---|---|---|---|---|---|
| Levine et al 1973 | 28 | .79+ | GVN | 38 | .79+ |
| Levi et al 1973* | 28 | .89 | GVN | 21 | .48 |
| Schimpff et al 1975 | 21 | 3 | GVN | 19 | 1.7 |
| Klastersky et al 1974 | 13 | 1.38 | BNNP + Gentamicin | 14 | 1.42 |
| Dietrich et al 1977* | 44 | 1.02 | BNNP | 42 | .83 |
| Keating and Penington 1973 | 66 | .6 | Framycetin | 38 | .76 |
| Storring et al 1977* | 49 | .98 | FRACON | 46 | .52 |
| Rodriguez et al 1978* | | | PA[1] | 55 | .53 |
| | | | SA[2] | 90 | .49 |
| Rozenberg-Arska et al 1980 | 20 | 1.2 | TMP/SMX | 18 | .44 |
| Sleijfer et al 1980 | 55 | .69 | multiple[5] | 58 | .16 |
| Buckner° et al 1978* | 44 | 1.39 | GVN plus | 45 | .44 |

+ severe infections only  * both groups in protective isolation
° marrow transplant patients

PA[1], SA[2] see overleaf

Table 7b

PA[1]

| | |
|---|---|
| Paromomycin | 1.5 gms/day |
| Polymixin B | 200 mgs/day |
| Vancomycin | 750 mg/day |
| Nystatin | 10 x $10^6$ units/day |
| 5FC | 1.5 gms/day |
| Cephalexin | 1.5 gms/day for 5 days |
| Chloramphenicol | 1.0 gms/day for 5 days |
| Ampicillin | 3 gms/day for 5 days |
| Clindamycin | 1.2 gms/day for 5 days |

SA[2]

| | |
|---|---|
| Cephalothin | 8 gms/day for 5 days |
| Colistin | 200 mg/$m^2$ for 3 days |
| Chloramphenicol | 2 gms/day for 5 days |
| Amphotericin B | 10 mg/day for 2 days |
| Ampicillin | 2 gms/day for 5 days |
| Gentamicin | 30 mg/$m^2$/day for 6 days |
| Clindamycin | 1.2 gms/day for 6 days |
| Amphotericin B | 10 mg/day for 2 days |

For other regimens - see tables 4 and 5b

The one study of marrow transplant patients (Buckner et al 1978) showed that leukaemia patients transplanted in relapse suffered 50% more infections than those transplanted in remission.

Bacteraemias

Table 8 shows the number of bacteraemias per patient with different antimicrobial prophylaxis regimens. The degree of neutropenia varied but diagnoses and therapy were similar except in the report by Gurwith et al (1978) who did not quote the diagnosis. All authors found a reduction in bacteraemia with their regimens but results were improved by added protective isolation. With this, GVN and FRACON ± co-trimoxazole all gave between .09 and .16 bacteraemias per patient. Neither Sleijfer et al (1980) nor Rosenberg-Arska et al (1980) quote the degree or duration of neutropenia, but both showed useful results in preventing bacteraemia during remission induction of AML.

The multiple drug regimen of Guiot and Van Furth (1977) given to 9 transplant patients seemed effective. Winston et al (1978) gave routine parenteral amphotericin to their second group of transplant patients having seen 16 fungaemias in the first group, probably associated with 'SCARI', their very toxic pre-conditioning regimen.

Buckner et al (1978) took some care assessing compliance with the prophylactic antimicrobial agents and showed how this affected the incidence of bacteraemia in their transplant patients.

| Compliance | Patients | Bacteraemias | Bacteraemias per patient |
|---|---|---|---|
| good | 12 | 2 | .17 |
| "average" | 24 | 5 | .21 |
| bad | 8 | 3 | .38 |

Schimpff et al (1978) found that compliant patients had a bacteraemia rate of 0.3/100 days whereas the poorly compliant had a rate of 2.1/100 days. Compliance adds a further variable to assessments of the antimicrobial regimens. On the basis of infections per patient and the incidence of bacteraemia, there is little to choose between the GVN and FRACON regimens, even when a protected environment is included. The combination of a protected environment with these prophylactic antibiotics

Table 8

COMPARISON OF ANTIMICROBIAL REGIMENS
Influence on Bacteraemia

| Author | Control group bacteraemias/ patient | REGIMEN | Prophylactic antibiotic group bacteraemias/ patient | Antimicrobials bacteraemias /patient with additional isolation |
|---|---|---|---|---|
| Levine et al 1973 | .25 | GVN | .21 | .09 |
| Schimpff et al 1975 | .76 | GVN | .37 | .16 |
| Klastersky et al 1974 | .61 | BNNP+G | .5 | .63 |
| Keating and Penington 1973 | 1.28 | FRACON | .87 | |
| Storring et al 1977 | .37 | FRACON | .13 | |
| Gurwith et al 1978 | .21 | TMP/SMX | .02 | |
| Enno et al[1] 1978* | .31 | TMP/SMX | .14 | |
| Sleijfer et al 1980 | .15 | multiple | .05 | |
| Rozenberg-Arska et al 1980 | .3 | TMP/SMX | .17 | |

MARROW TRANSPLANT PATIENTS
(Acute leukaemia and aplasia)

| Author | Regimen | Protected environment | Patient numbers | No. of Bacteraemias | Bacteraemias /patient |
|---|---|---|---|---|---|
| Guiot and Van Furth 1977 | Multiple | LAF | 9 | 1 | .11 |
| Winston et al 1978 | GVN<br>GVN+A(IV) | BN<br>BN | 60<br>18 | 48(16 fungal)<br>6(1 fungal) | .80<br>.33 |
| Buckner et al 1978 | -<br>GVN plus | BN<br>LAF | 44<br>45 | 23<br>10 | .52<br>.22 |

*PE used in both groups
LAF laminar air flow
BN Barrier nursing

[1] FRACON given to both groups

For details of regimens see tables 4 and 5b

is more effective but the results with co-trimoxazole are encouraging.

Patients developing no infection

This is not recorded sufficiently frequently to make any comparison of antimicrobial regimens per se, but the combination of protective isolation with these prophylactic antimicrobial agents is highly effective.

| Author | Patients with no infection/total patients | Patients in protective isolation taking GVN with no infection/total patients |
|---|---|---|
| Bodey et al 1971 | 18/66 (27%) | 15/33 (45%) |
| Levi et al 1973 | 8/28 (29%) | 14/21 (67%) |
| Schimpff et al 1975 | 2/21 (10%) | 8/24 (33%) |

24% of control patients had no infection compared with 47% who were in protective isolation and receiving GVN ($p < .05$). 61% of AML patients taking co-trimoxazole in the ward received no additional antibiotics compared to 15% of control patients (Rozenberg-Arska et al 1980). This data is not reported in other trials and is worthy of further study.

Deaths from infection

Table 9 compares the deaths from infection occurring with each regimen, comparing control patients with those in protective isolation and receiving prophylactic antimicrobial agents. Overall 107 (22%) of 484 patients in the 'control' arms died of infection compared with 61 (12%) of 527 ($p < .001$) who received prophylactic antimicrobial agents in protective isolation.

Whilst table 9 does not compare only the effects of prophylactic antimicrobial agents in preventing death from infection, the numbers of patients receiving the different regimens within a protected environment does allow some comparison. The GVN reports are mainly from the early 1970's but the proportion of protected patients dying of infection has remained fairly constant in each study. Summating the deaths in each group gives the following results

Table 9

COMPARISON OF ANTIMICROBIAL REGIMENS

Deaths from infection

| Author | NO PROPHYLACTIC ANTIMICROBIALS | | PROPHYLACTIC ANTIMICROBIALS | | |
|---|---|---|---|---|---|
| | Patients | Patients died of infection | REGIMEN | Patients | Patients died of infection |
| Bodey et al 1971 | 66 | 14 (23%) | GVN* | 33 | 3 (9%) |
| Levine et al 1973 | 28 | 6 (21%) | GVN* | 22 | 0 (0%) |
| Levi et al* 1973 | 28 | 6 (21%) | GVN* | 21 | 3 (14%) |
| Yates and Holland 1973 | 31 | 6 (19%) | GVN* | 20 | 2 (10%) |
| Schimpff et al 1975 | 21 | 11 (52%) | GVN* | 24 | 3 (13%) |
| Schimpff et al 1978 | | | | 42 | 5 (12%) |
| | 174 | 43 (25%) | | 162 | 16 (10%) |
| Rodriguez et al 1978 | 82 | 23 (28%) | PA ± PE | 54 | 13 (24%) |
| | 63 | 8 (13%) | PA ± PE | 90 | 18 (20%) |
| Klastersky et al 1974 | 13 | 4 (31%) | BNNP + G* | 16 | 6 (38%) |
| Dietrich et al 1977 | 51 | 8 (16%) | BNNP* | 42 | 4 (11%) |
| Storring et al* 1977 | 49 | 12 (24%) | FRACON* | 46 | 2 (4%) |
| Enno et al 1978 | | | FRACON* | 16 | 2 (13%) |
| | | | FRACON + TMP/SMX* | 14 | 0 (0%) |
| Watson and Jameson 1979 | | | NEOCON* | 34 | 0 (0%) |
| Sleijfer et al 1980 | 52 | 9 (17%) | Multiple | 53 | 0 (0%) |

Details of regimens - see tables 4 and 5b

*protected environment (PE) in use

% of patients dying from infection

| | No. of patients | control | protected environment plus prophylactic antimicrobials |
|---|---|---|---|
| GVN | 336 | 25% | 10% |
| BNNP | 122 | 19% | 17% |
| FRACON/ NEOCON | 145 | 24% | 4% |

If the earliest GVN study (Bodey et al 1971) is excluded the percentage dying of infection while receiving GVN remains at 10%. The results of the FRACON type regimen are better than those for GVN within the difficulties of this comparison. There is nothing to recommend the BNNP regimen or the multiple systemic antibiotic regimen of Rodriguez et al (1978). However none of 67 patients receiving co-trimoxazole died of infection.

Cost

| Author | Regimen | Cost |
|---|---|---|
| Bodey et al 1974 | GVN | £42.11 per day |
| Schimpff et al 1975, 1978 | GVN | £60.78 per day |
| Storring et al 1977 | FRACON | £5.12 per day |
| Watson and Jameson 1979 | NEOCON | £2.84 per day |

The difference in cost (MIMS 1979) between the regimens of Schimpff et al (1978) and Storring et al (1977) was £54 per patient per day which would almost pay for two additional staff nurses per patient each day. This is the most substantial difference between any of the regimens.

Conclusions

In summary, the three most reported regimens are GVN, FRACON/NEOCON and BNNP. BNNP is the least effective in a number of assessments. There is little to choose between GVN and FRACON as far as elimination of pathogens or prevention of bacteraemia. FRACON is probably superior to GVN in reducing the number of fever days and the days when therapeutic antibiotics were given. GVN is no better than other regimens in preventing infections, but FRACON is superior in preventing deaths from infection. When a protected environment is used with antibacterial agents, results are improved.

As GVN is not superior to FRACON, and costs considerably

more there is no reason to recommend GVN. On limited evidence, the formulation of FRACON can be altered and the dose per day halved without detriment (Watson and Jameson 1979). Co-trimoxazole regimens are even cheaper and may be more effective. Until these are evaluated, NEOCON (Watson and Jameson 1979) must be the recommended prophylactic regimen, but urgent evaluation of co-trimoxazole is required.

Unwanted effects of antimicrobial prophylaxis

Oral antimicrobial regimens are of little use if not tolerated by the patients. The antimicrobial agents may be directly toxic, may interact with chemotherapy or select a population of antibiotic resistant organisms. Interpreting anorexia, nausea and vomiting with these regimens is difficult owing to the concurrent chemotherapy and reports of diarrhoea and abdominal pain are much modified by the use of codeine phosphate and similar agents.

The rotating regimen of Priesler et al (1970) was poorly tolerated with frequent anorexia, nausea and vomiting. All patients had mild diarrhoea as did many taking GVN (Priesler et al 1970; Bodey et al 1974). A few patients had abdominal cramps and severe diarrhoea. Bodey et al (1969) found GVN as well tolerated as paromomycin, polymyxin and vancomycin. Even so, 15% of patients do not tolerate GVN (Bodey et al 1972; Levi et al 1973). Bodey et al (1969) found nausea on one third of the days and vomiting on one day in nine. Rodriguez et al (1978) found one-third of their patients receiving the complex prophylactic regimen (table 7 ) had nausea and epigastric pain. Although these effects may seem trivial, they are an additional burden for the patient and interfere with adequate nutrition.

Some intolerance to all regimens is reported, the effects usually being as described for GVN. Storring et al (1977) found 10% could not tolerate FRACON, and Watson and Jameson (1979) using half doses found an equal percentage intolerant of NEOCON. Co-trimoxazole caused a rash in about 12% of patients (Gurwith et al 1978). Schimpff et al (1978) found that GVN was taken on 82% of days. Buckner et al (1978) using GVN $\pm$ polymyxin and paromomycin found that only 25% of patients took all their antimicrobial regimen and that one patient in six

took less than 10% of the prescribed amount. This latter group had more than twice as many severe infections as the former group. After total body irradiation prophylactic antimicrobials may not be tolerated for a few days (Guiot and Van Furth 1977), and the Leiden group currently give co-trimoxazole intravenously if the oral antibiotics are not tolerated.

There are also positively harmful effects of the antimicrobial regimens. Four of 90 patients receiving prophylactic systemic antimicrobials including amphotericin B and colistin developed acute renal failure with three deaths. Six of 145 receiving prophylactic clindamycin developed necrotising enterocolitis, of whom two died. (Rodriguez et al 1978). Keating and Penington (1973) had one case of necrotising enterocolitis in 38 patients receiving framycetin. Gentamicin absorption from the GVN regimen is usually insignificant although Priesler et al (1970) found half their patients to have detectable serum levels. One with radiation enteritis had a serum level of 0.53 μg/ml and excreted 10% of the administered gentamicin in the urine. Levi et al (1973) found detectable serum gentamicin on a third of occasions. Neomycin and framycetin are directly toxic to the gut causing villous changes and mice tolerate methotrexate less well when also receiving neomycin (Zaharko et al 1969), probably because neomycin reduced the absorption of methotrexate and also its degradation to non-toxic metabolites. Neomycin might therefore be an unwise choice where gut toxicity is the therapeutic limiting factor as with high dose melphalan therapy.

Another problem is resistant organisms especially when the oral antibiotics include those usually used in a therapeutic role. Bodey et al (1972) found 22% of bacterial isolates from their patients receiving prophylaxis were resistant to the absorbable antibiotics they used (tetracycline, chloramphenicol, doxycycline and ampicillin). Klastersky et al (1974) reported that one-third of organisms causing infection in their patients were resistant to the oral prophylactic kanamycin and polymixin B used and the in-vitro sensitivity to gentamicin was also greatly decreased. In Baltimore (Schimpff et al 1973) gentamicin resistant pseudomonads were an occasional problem but by 1978 Schimpff had to use amikacin as first line aminoglycoside

therapy in the neutropenic febrile patient. Although the GVN regimen was not solely responsible its use outside a protected environment must have played a part.

Interrupting prophylactic antimicrobials during neutropenia in hospital is also unwise. Eight of 43 patients unilaterally stopped taking GVN (Schimpff et al 1975). Of two in protective isolation one died of infection and of six patients not in protective isolation four died of infection, three due to a hospital acquired pseudomonas spp. This further argues in favour of protective isolation when prophylactic antibiotics are being used.

For a variety of reasons all the regimens seem not to be tolerated by 10-15% of patients. The use of antimicrobials with known highly toxic effects on the gut seems unwise and the use of potentially powerful therapeutic antibiotics as routine prophylaxis has little to commend it as a policy.

Recontamination after suppression of microbial flora

Protective measures adopted during leukaemia remission induction or marrow transplantation are usually withdrawn when the neutrophil count is in excess of $1 \times 10^9/l$. In SCID, withdrawal of protective measures must await objective signs of a functioning transplant. If acute graft-versus-host disease (GVHD) is considered to be related to the quantity or nature of micro-organisms in the gut, protective measures will be indicated for 60 days, after which the incidence of GVHD is less although 30 days may be sufficient (Vossen 1980).

Recontamination is necessary only for an axenic patient. Patients undergoing leukaemia remission induction are not axenic and simply stopping their oral antimicrobial agents leads to rapid alimentary recolonisation. Bodey and Rosenbaum (1974) found half the organisms present in the stools between 3 and 17 days after stopping GVN had been present before oral antimicrobials commenced.

Composition of stool flora of 42 patients after stopping GVN

| | Total Isolates | previously isolated | apparently new acquisitions |
|---|---|---|---|
| aerobes | 202 | 112 (55%) | 90 |
| anaerobes | 37 | 19 (51%) | 18 |
| fungi | 11 | 5 (45%) | 6 |

Kliebsiella spp, lactobacilli spp., proteus spp., citrobacter spp. and streptococci spp. were the most common bacteria to re-appear. Enterobacter, esch coli, proteus spp. and clostridia spp. were the most common apparently new acquisitions. One-third of the micro-organisms which persisted during decontamination vanished on re-emergence of the previously suppressed flora.

This occult viable load of micro-organisms makes it difficult and probably unnecessary to actively recontaminate the gut. The patient has previous experience of these organisms and should have some appropriate cellular or humoral immunity. Their growth following withdrawal of oral antimicrobials militates against overgrowth by new exogenous organisms and sending the patient home directly from isolation means that such organisms as are acquired will not be antibiotic resistant hospital flora. If fungal overgrowth is thought possible, oral antifungal agents should be continued.

Marrow transplant recipients however take up to 12 months to recover their immunity or longer if GVHD occurred (Witherspoon et al 1976) and their allogeneic graft may not be able to produce a rapid secondary response. If prophylactic cyclosporin-A is being given, the graft may be tolerant of new antigens. Child sibling donors usually share a home environment and their microbes with the patient so this tolerance may not be a real problem.

Septrin, given after grafting for pneumocystis prophylaxis, suppresses stool microflora and may therefore reduce the inoculum of a new organism to below the level required for colonisation. However, some centres actively recontaminate after transplantation, giving the organisms either by mouth or per rectum.

Fresh yoghurt or subcultures of fresh yoghurt have been used (Schwartz and Perry 1966) as have the faeces of pathogen-free mice. Another approach is to use organisms selected for their low pathogenicity and high antibiotic susceptibility either from the donor's stools (Ramsoe et al 1978), or the patient's original flora preserved in liquid nitrogen from before decontamination. Additional anaerobes may be required

as these survive poorly in liquid nitrogen. O'Reilly et al (1977) at Sloan-Kettering uses a mixture of diphtheroids, neisseria spp., lactobacilli, clostridia, bacteroides spp. (not fragilis) and peptostreptococci until $10^8$ organisms/gm are present in the stool. 10% of their patients become transiently febrile with this regimen though none developed clinical infections.

The value and the ease of recontamination has been overstated. For the non-axenic patient to go straight home from isolation, stopping their oral antibiotics as they leave hospital is the simplest policy. For axenic infants it would be wise to establish a low-grade pathogen alimentary flora of strep. faecalis, bacteroides spp. and lactobacilli with staph. epideridis applied liberally to the skin before leaving isolation.

## INFECTION AS A TRIGGER FOR GRAFT-VERSUS-HOST DISEASE

In addition to the benefits that a protected environment with oral prophylactic antimicrobial agents gives in preventing infection in patients undergoing marrow transplantation, the recipients of an allograft are also at risk from graft-versus-host disease (GVHD). As yet there is limited evidence in man that GVHD is triggered by infection but a number of anecdotal reports and impressions suggest that microbial flora have a role in the development of this problem and animal evidence of the relationship between GVHD and micro-organisms cannot be dismissed lightly.

### Animal Evidence

Table 10 shows the results of Van Bekkum et al (1974) who compared the stool microbial status of recipient CBA mice with their survival when given 900 rads total body irradiation followed by intravenous spleen and bone marrow cells from conventional H-2 non-identical C57 Bl mice. The conventional recipient mice had normal laboratory mouse flora; the germfree (GF) mice were strictly speaking gnotobiotic, as a number of vertically transmitted viruses exist. The "colonisation resistant" (CR) mice had a stool anaerobic flora, the presence of which greatly increased the number of aerobes required to colonise their bowel (Van der Waaji et al 1971). These CR mice were achieved either by contaminating GF mice with flora from a CR mouse or by decontaminating conventional mice with 5 gms of neomycin, streptomycin and bacitracin, and 100 mg pimaricin added to each litre of drinking water.

From table 10, it is clear that the germfree state conferred great protection against death from GVHD, as it had been previously shown (Wilson 1963) that without grafting only 5% of germfree and 6% of conventional mice exposed to 900 rads would survive. Most GF mice suffered minor GVHD but they all recovered in similar recent experiments (Heidt et al 1979) and similar results have been found in other rodents (Table 11). The results of grafting CR mice are sufficiently similar (table 10 ) to those of grafting GF mice for an intrinsic immunological difference resulting from the GF state to be unlikely to be the explanation.

Van Bekkum et al (1974) contaminated mice chimeras which

Table 10

RESULTS OF GRAFTING C57 B1 MARROW AND SPLEEN CELLS INTO CBA MICE OF DIFFERING MICROBIAL STATUS

| Microbial status of recipient mouse | Survival at 90 days |
|---|---|
| conventional | 5% |
| Colonisation resistant | |
| from germfree | 90% |
| from conventional | 93% |
| germfree | 100% |

deaths were almost all due to GVHD

GF AND GF/CR CBA/C57 B1 CHIMERAS

CONTAMINATED WITH CONVENTIONAL FLORA AFTER GRAFTING

| Time post-graft of contamination | 80 day survival post contamination |
|---|---|
| 8 days | 0% |
| 14 days | 0% |
| 26 days | 60% |
| 40 days | 92% |
| 60 days | 74% |
| 80 days | 100% |
| 100 days | 100% |

Table 11

## INCOMPATIBLE RODENT BONE MARROW GRAFTS

(Pollard et al 1976)

Mice

| Donor | Recipient and microbial status | Post graft contamination | Result |
|---|---|---|---|
| C3H/He ($H2^k$) | DBA/2 ($H2^d$) GF | Staph. epidermidis<br>B. megaterium<br>Serratia spp. | survived |
| DBA/2 ($H2^d$) | AKR ($H2^k$) GF | Lactobacillus casei<br>Strep. faecalis<br>Bacteroides spp.<br>Clostridium difficile | persistent carriers of organisms without detriment |
| | | Sal. paratyphi B | died |
| C3H | Haas GF | - | No GVHD |
| DBA/2 | AKR CR | - | survived |
| DBA/2 | AKR GF | LAF at +30 days clean conventional room at +60 days | survived no GVHD |
| SJL/J ($H2^s$) | C3H ($H2^k$) GF | LAF at +30 days clean conventional room at +60 days | 35% survived 140+ days in room |

Rats

| | | | |
|---|---|---|---|
| GF Wistar | Sprague Dawley GF | gram positive spore formers | survived |
| Wistar conventional | Sprague Dawley conventional | - | died of GVHD |
| Rats | Mouse GF | - | died of GVHD |

GF - gnotobiotic

Decontaminated - conventional animals treated with antibiotics

were GF or CR derived from GF at different times after grafting and observed GVHD. They contaminated the GF group with CR flora for 4 days before transfer to a conventional mouse room to avoid rapid death which sometimes follows sudden conventionalisation of GF mice (Jones et al 1971). Table 10 shows that stable chimeras can be contaminated and live in a normal mouse room without developing GVHD providing this contamination occurs after a certain date. Another group - CR derived from conventional mice using antibiotics - were reconventionalised at 66 days with 67% surviving the next one hundred and fifty days but dying with what seemed to be low-grade GVHD. This result is not dissimilar to the group in table 10 reconventionalised at day 60. This group (CR derived from conventional) mimics human aplastic anaemia and acute leukaemia transplant decontamination but the axenic infant with severe combined immune deficiency could be microbiologically analogous to the germfree mouse.

Table 11 shows a summary of the organisms used to contaminate germfree rodents after H2 incompatible marrow transplants. No details of the timing of the contamination were recounted but the final results are similar to those of Van Bekkum et al (1974). There was no evidence that congenital viruses induced GVHD, and in addition no one particular species or class of organism has been reported which will trigger GVHD. Likewise endotoxin has not proved to be a trigger. Most groups of GF recipients had high survival rates following an incompatible transplant but even the GF state would not permit the xenogeneic transplant of rat marrow intc mouse.

Van Bekkum et al (1977) introduced further mouse evidence of the role of micro-organisms in gut GVHD. Germfree and conventional C57 Bl x CBA F1 mice had fetal F1 gut implanted subcutaneously. Total body irradiation was followed by marrow and spleen cell transplantation from a CBA donor. Conventional recipient mice died at 20 days of GVHD. Killing the conventional mice at earlier times showed alimentary tract lesions and also damage to the implanted F1 fetal gut. Germfree recipient mice killed at the same time after grafting showed some lesions of gut GVHD in their alimentary tracts but comparatively much less damage to the F1 fetal gut implant. The derived hypothesis

was that gut microfloral antigens gained access via minor GVHD lesions, cross-reacted with host tissue antigens and induced a considerable secondary response in the transplanted tissue, which then reacted with recipient tissues. Decontamination of the gut removed these gut antigens and so in the presence of mild GVHD, the secondary response did not occur.

Human evidence

Evidence in humans is accumulating slowly, one difficulty being the lack of axenic humans. No series of transplants in axenic SCID infants has been reported, but GVHD has been related to the gut decontamination status of other transplant patients (table 12 ). The data of Vossen (1980) is one of the few reports and involves only children with aplasia. There is no doubt from these three sources that stool microbial suppression is advantageous in allogeneic transplantation for aplastic anaemia. Table12a shows GVHD in eighteen of twenty-two recipients whose stool micro-organisms were not suppressed, compared with one of sixteen who had suppression of stool microflora. The majority of these aplastic patients had specific proof of engraftment.

This benefit is not seen in leukaemia patients undergoing transplantation (table12b) even in those who received cyclosporin-A as prophylaxis against GVHD. Their stools were no different in content of micro-organisms (Watson and Jameson 1979) from those of patients who had received methotrexate prophylaxis. The major difference between the pre-conditioning regimens in aplasia and leukaemia is the use of 1000 rads total body irradiation in leukaemia. Does this induce gut changes that allow microbial antigens access at the time of, or independent of, minor GVHD changes in the gut, thereby sensitising the transfused immuno-competent cells with a secondary response against tissue antigens resulting?

If the relationship between GVHD and stool microbial suppression is confirmed in aplasia then new impetus will be given to antimicrobial regimens both at the time of and after transplantation.

# HUMAN MARROW TRANSPLANTS, GVHD AND STOOL MICROBIAL STATUS

Table 12a

Transplants for aplastic anaemia

| Author | No. of patients | stool status | No. with GVHD |
|---|---|---|---|
| Guiot and Van Furth 1977 | 2 | suppressed[1] | 0 |
| | 4 | not suppressed | 2 |
| Buckner et al 1978 | 4 | suppressed[2] | 0 |
| | 12 | not suppressed | 11 |
| Vossen 1980 | 10 | suppressed[1] | 1 |
| | 6 | not suppressed | 5 |

Table 12b

Transplants for acute leukaemia

| Author | No. of patients | stool status | No. with GVHD |
|---|---|---|---|
| Buckner et al 1978 | 5 | suppressed[2] | 4 |
| | 24 | not suppressed | 8 |
| Royal Marsden Hospital | | | |
| methotrexate prophylaxis | 5 | suppressed[1] | 4 |
| | 9 | not suppressed | 4 |
| cyclosporin-A prophylaxis | 12 | suppressed[1] | 3 |
| | 9 | not suppressed | 2 |

[1] complete and sustained suppression of all faecal flora

[2] neither enterobacteriacae nor candida grown until at least 30 days after grafting

## 2. BONE MARROW TRANSPLANTATION FOR INFANTS AND CHILDREN AT TWO LONDON HOSPITALS

### Introduction

Work at two centres is reported in this thesis. Originally the Co-operative Group for Bone Marrow Transplantation in London decided that infants with immune deficiencies would receive their transplant at Westminster Children's Hospital and children with leukaemia would be transplanted at the Royal Marsden Hospital. Children with aplastic anaemia would be grafted at either centre or at the Hammersmith Hospital. In the course of time, the Royal Marsden Hospital transplant team concentrated on acute leukaemia and subsequently on only acute myeloid leukaemia, referring children with acute lymphoblastic leukaemia to Westminster Children's Hospital. This latter arrangement occurred at the same time as I moved from Westminster Children's to the Royal Marsden Hospital and is the reason why there is no separate chapter on the transplantation of children with acute lymphoblastic leukaemia.

Both the Royal Marsden Hospital and Hammersmith Hospital had purpose build leukaemia or anaemia units. Westminster Children's Hospital had no special facilities to compare with the Bud Flanagan Unit at the Royal Marsden Hospital which contained 10 single cubicles each with filtered positive pressure air and an ante-room in which staff washed and changed. At Westminster Children's Hospital, a general paediatric hospital in central London, there were no specific isolation facilities and consequently, amongst all the arrangements required to conduct a bone marrow transplant programme, an isolation system had to be created and techniques of anti-microbial prophylaxis had to be developed. It is these problems which are now described and discussed.

## A) PROTECTED ENVIRONMENTS AND PROPHYLACTIC ANTIMICROBIAL AGENTS

### The Establishment, Use and Evaluation of Vickers-Trexler isolators in Westminster Children's Hospital

#### The decision to use isolators

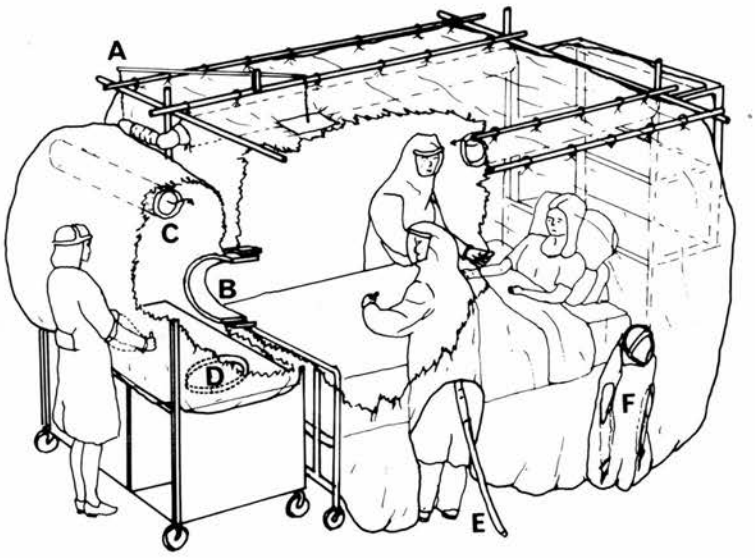
Westminster Children's Hospital was opened in 1908 and had 72 beds and 26 cubicles in 1975. Twelve cubicles were specifically for infants. The 14 other cubicles, divided between three wards, were not allocated to specific consultants and contained medical or surgical patients as need dictated. These cubicles were occupied 85-90% of the time. They had no ante-rooms so all hand-washing and gowning by staff was performed in narrow corridors. The sinks had elbow-operated taps with a single water jet hitting the plug-hole directly. The surrounding plaster was old and defective in many areas near the sinks and the cubicles contained numerous pipes and ducts which made them difficult to clean. The floors were of pervious cork and glass in the corridor windows and door made communication with confined children difficult.

These cubicles were unsatisfactory for protecting a child with prolonged neutropenia and upgrading programmes were severely hampered by financial restraints. The demand for cubicles was such that the three month stay envisaged for a child undergoing bone marrow grafting would seriously reduce the availability of cubicles for other children. An alternative form of protection which supplied practical effective isolation and at the same time required no structural alterations or permanent site in the hospital was required and it was with these aims that a Vickers-Trexler Mark I isolator was obtained using charitable funds.

#### The Vickers-Trexler Mark I isolator

Earlier models of patient isolator (Robertson et al 1968, Barnes and Tuffrey 1968) bore only a superficial resemblance to the model described by Trexler et al (1975) and shown in the cut-away diagram (fig. 1 ) on the next page.

The isolator consists of two separate polyvinyl chloride (PVC) bags, called envelopes, which are linked by a communicating port. The main patient envelope hangs from supporting




Bed isolator showing patient in bed and two half suits in use. A nurse is working in supply isolator on left. A = Control mechanism for regulating pressure in isolator. B = Patient entry port. C = Air filter for pressurising supply isolator. D = Downflow supply port. E = Air supply to half suit. F = Unoccupied half suit.

Fig. 1 The Vickers Trexler Isolator (Mark I)

rods joined by triclamps, and reaches the floor only on the patient's left side. The standard NHS bed and other furniture is outside the envelope and the mattress fits in an external pocket on the outside of the envelope base. Foam rubber separates and protects the PVC from the bed springs. The bedding is inside the isolator and the way the PVC mattress pocket is attached allows the bed to be made in a normal manner. Medical and nursing equipment is inside the envelope , stored on shelves and racks. The supply envelope, so-called because it has a hatch where supplies enter, is at the foot of the bed and is also supported on a framework (not shown above). There is a foot operated hatch through which sterile items may enter the isolator. An exit port for removing waste is fitted to the patient envelope. The envelopes are kept at a positive pressure by HEPA filtered air supplied by a blower. The flow of filtered air entering the isolator is controlled by a hand-valve and a pivot system with a ball valve automatically adjusts the amount of air actively extracted from the isolator by the same blower,

thus maintaining the correct positive pressure within the isolator.

Access to the patient is provided by three half-suits which are part of the isolator wall and a number of sleeves with gloves, spaced at intervals round the isolator. The half-suits cover only the upper half of the body and have a visor of firm clear plastic. Each has four arm sleeves with gloves. A lightweight air waistcoat is hung round the attendant's neck and this delivers air from a small additional blower to the attendant. It takes 15 seconds to get into a half-suit and no protective clothing is required.

A fuller description of the isolators and how to use them to care for severely neutropenic or immunosuppressed children is given in appendix I. This appendix is my tape-slide teaching aid which was used to familiarise new medical and nursing staff with isolator care. It is purely descriptive and is relevant only to the techniques of paediatric isolator care.

The Vickers-Trexler Mark I isolator tents were designed for adults and were not ideal. To care adequately for children, it was necessary to modify the design and construct some new models. Adult-sized envelopes were inappropriate for infants, so small animal isolators were adapted for this purpose. For toddlers an adult-sized envelope was altered to completely enclose a standard NHS cot. A transit isolator to allow isolated children to be moved around the hospital or between hospitals was also designed. Examples of these different isolators are shown in appendix I. The supporting framework was standardised and almost all the hardware became inter-changeable between the different isolators.

Isolator modifications introduced at Westminster Children's Hospital

Modifications of our exclusion isolation system had to be compatible with the Coppets Wood Isolation Hospital's isolators, which are used for the containment of dangerous fevers. Thus the improvements and modifications introduced would benefit patients requiring either exclusion or containment isolation. These developments were performed in conjunction with Vickers Medical and Mr. P.C. Trexler of the Royal Veterinary College.

Technical modifications

1. Single piece demountable and frameworks

Each end framework from which was hung the patient envelope consisted of eight separate rods with additional shelving or a panel including the ports joining the patient and supply envelopes. These rods had to be assembled and clamped together so the ports of the supply envelope and the patient envelope were at the same height. These rods were now welded together making each end-frame a single unit with an adjustable position for the joining port. This saved 3 hours each time an isolator was assembled.

2. Foot operated side entry port

The nurses had to crouch to use the downflow hatch to enter supplies into the isolator. The hatch cover was also potentially contaminated on its lower side. A side-entry with bivalve flaps opened by a foot pedal was easier for the nurses to use and the bivalve flaps effectively sealed the port. Passing in heavy items, e.g. sheets, was a little more difficult as these were received horizontally at arms-length rather than vertically. A side-entry port allowed the same isolator to be converted for containment isolation (Fradd 1979) which was not possible using a downflow port. A further development must be a sloping entry port angled so that only one nurse is required to enter supplies.

3. A separate exit port and waste bag

This was re-introduced to allow large volumes of water to be removed by suction and the presence of a waste bag meant many fewer openings of the entry port. This bag needed changing daily and this could be performed without breaking isolation. Urgent specimens, e.g. blood samples were removed via the entry port.

4. A dispersal system for the sterile air

Fitted to the ceiling of the patient envelope this reduced filter flow noise and stopped draughts from the sterile air entering the isolator.

Some minor technical modifications are still required. The half-suit air blower should have additional silencing (p 75 ). The air

waist-coats should be more durable and the visor/flexible PVC half-suit seam should be strengthened. In addition, such alterations as are wished by those caring for patients in containment isolation or responsible for their transport will have to be incorporated into the exclusion system to maintain industrial compatibility.

Modifications related to patient care

The size, shape, position and nature of what was required, and the thickness of the PVC, were discussed with industry. The prototypes were assessed, further alterations made and a number of the ideas are now incorporated as standard on all the most recent (Mark 3) isolators.

1. Four arm-sleeves on each half-suit

These allowed two different pairs of gloves to be available in each half-suit. The spare sleeves made glove changing easier and this could be done electively at a less busy time. If surgical gloves were required e.g. to allow a venous cut-down, they were replaced at leisure by normal gloves. For routine work, different sizes allowed the nurses and doctors to have gloves which fitted, important if an hour was being spent in a half-suit or 25 gauge scalp needles were being used for children whose veins were becoming increasingly difficult. 'Dumor' size $6\frac{1}{2}$ and 8 gloves were most usually fitted.

2. X-ray tube drive-in sleeve and separate cassette sleeve

Before this development, x-rays were taken from one side of the isolator with the film on the other side. This meant the patient had to sit transversely across the bed and the almost fixed distance did not make for the usual high quality radiographs. PVC, except at a seam, did not interfere with the picture obtained. The drive-in sleeve for the x-ray head fitted model GEC MX4 mobile, a standard NHS portable x-ray unit. The sleeve allowed full vertical ranging of the tube and the length of the sleeve allowed the x-ray head to cover all but the end foot of the bed. The x-ray head could be tilted inside the sleeve and a panel of clear PVC allowed the aiming light beam to be positioned correctly. The cassette sleeve

was 6 foot long (183 cms) and took cassettes up to 13 x 17 inches (35 x 43 cms). The sleeve length allowed the cassette to be placed in any required position.

This combination of tube and cassette allowed all plain x-rays including decubitus views of the chest and abdomen. Contrast films, e.g. confirming the position of a non-opaque long intravenous catheter, intravenous pyelography or a barium meal (using sterile barium) were also performed as were ultrasound studies.

The x-ray/weighing bag in the infant (cot) isolator was integral with the isolator and made of clear PVC. The neck was bunched and tied when not in use to prevent the infant having access to the bag. With a table placed below the bag and the x-ray film between the bag and table the infant was x-rayed with the tube shooting vertically downwards. Similarly, the infants were weighed on scales placed under the bag.

All these techniques were performed with hospital staff wearing their everyday clothes. Apart from hand washing there were no sterile precautions required.

3. Refrigerator sleeve

This was designed to fit inside the Electrolux 120, a standard ward fridge, and the sleeve fitted in continuity with the supply envelope. Although effective, changes are needed if ice is required, but 6$^{o}$C was readily achieved. The refrigerated part of the sleeve held 18-24 hours supply of infant feeds, 8 tins of coca-cola or similar drinks. The refrigerator fitted below the work surface of the supply isolator.

4. Additional storage space, sleeves and gloves

A number of shelves and storage racks were added and additonal glove sleeves were positioned round the envelope at the most useful sites, including one to allow toys to be picked up from the floor. Several were placed near storage areas so items could be easily taken from a shelf without entering a half-suit. A long sleeve at the head of the isolator allowed a telephone hand-piece to be used. The number was dialled through a nearby glove.

5. Additional cones

Many additional cones made of thin PVC were added, through which electric flexes, catheters or intravenous lines passed out of the isolator. This meant that numerous leads could be left in position. Leads for an ECG and a bell call were placed routinely. If necessary, leads supplying filtered oxygen or a suction facility were installed. Suction leads were left unplugged so the flow of air outwards through these left no damp dead space where organisms would proliferate. In the supply isolator, leads for an electric kettle, toaster, cooker and fan were routine. Ripple mattress leads were also fitted and suitable filters were included to prevent ward air contaminating the panels of the sterile ripple mattress.

Many of the children received intravenous feeding using Vamin-glucose, 10% glucose and occasionally Intralipid 10% in a rotating regimen. Although an intravenous facility was available wholly within the isolator using two extendible roof-top sleeves, it was simpler to manage intravenous therapy from completely outside the isolator. All drip tubing except the venous catheter was changed daily. From the isolator, a sterile anaesthetic extension set was passed out through a glove finger tip and taped firmly in position. This was connected to the fresh giving sets, filled with fluid and then connected to the venous line. The previous extension set was then drawn out of the isolator and discarded. The used glove finger tip was securely taped. Every fifth day the glove used for the intravenous line was changed.

These developments resulted in a patient care isolator which is the most complete available. As with many specialised systems there were a number of procedures to be learned but the discipline required was similar to that of an operating theatre. It was of course impossible for an isolator to operate distinct from the nursing staff and the hospital services but the effects of the isolator on these can only in part be separated from that of a marrow transplant programme.

Integration of the isolators with the hospital facilities

The earliest transplants were performed using conventional

reverse barrier techniques in the very inadequate ward cubicles briefly described on page 50. When the Vickers-Trexler isolator became available it was deliberately decided to incorporate the isolator both physically and for nursing purposes into the wards.

Infants were more appropriately nursed on the infant ward and not on a ward with older children, so the isolators operated on two separate wards depending on the age of the child (Figs. 2 and 3).

Nursing staff

When the General Nursing Council discontinued training solely Registered Sick Children's Nurses in 1975, Westminster Children's Hospital lost thirteen student nurses per annum. Concurrently the annual intake of qualified and student nurses for paediatric training was reduced from fifty to thirty. This reduction in real numbers of nursing staff caused considerable difficulty as, it must be clearly appreciated, the nurses caring for the bone marrow transplant children also had general ward responsibilities. For a general medical ward of up to 23 children, the nurse establishment was 21, of whom 12 were actually in post. For an infant ward of 16 patients, the establisment was 25 nurses of whom 15 were in post. The numbers and grades of staff established for and actually available are shown in Table 13. This situation was less than ideal so we employed two additional staff nurses paid from charity sources, as well as a packer to maintain the supply of sterile materials but some packing and all at weekends was carried out by ward nurses.

For nursing care, including cooking special meals, the 16 bed (10 isolation cubicles) leukaemia unit at the Royal Marsden Hospital has 31 established posts, all for post-graduate nurses. There are usually 25-28 nurses in post at any one time. Although the work is of a different nature in that unit, it is clear from my experience that a marrow transplant patient requires two to three times the usual nurse/patient ratio. Thus the employment of two additional staff nurses who also worked with the ward children ensured that the child undergoing a bone marrow transplant did not claim an unjustifiably large amount of the time of the hospital employed nurses. All the nurses including those specially employed came under the jurisdiction of the ward sister.

Fig. 2 VICKERS-TREXLER ISOLATOR ON A PAEDIATRIC WARD

Fig. 3 VICKERS-TREXLER ISOLATOR IN AN INFANT WARD

Table 13

CHILDREN'S MEDICAL WARD

20 beds* and one isolator

| Establishment 1978 | | Actual Average Numbers |
|---|---|---|
| Sisters | 2 | 2 |
| Staff Nurses | 6 | 3 + 2 ** |
| Enrolled Nurses | 1 | 0 |
| Nursery Nurses | 0 | 1 |
| "Learners" | 12 | 6 |

INFANT WARD

16 infants including 4 cot ITU area

| Establishment 1978 | | Actual Average Numbers |
|---|---|---|
| Sisters | 2 | 2 |
| Staff Nurses | 7 | 4 |
| Enrolled Nurses | 2 | 1 |
| Nursery Nurses | 2 | 2 |
| "Learners" | 12 | 6 |

* one isolator occupies 3 beds, i.e. reduction of 2 beds

** 2 staff-nurses specially employed

If "learners" cannot participate, on grounds that the work is too specialised, yet the work is inappropriate for inclusion in an intensive-care training course, then major difficulties with staffing levels will lie ahead.

## Other Hospital Services

### Bed availability

The bed isolator required 165 sq. ft. of floor space to be easily used though 120 sq. ft. was just manageable. Three ordinary beds had to be removed to position the isolator. The cot isolator required 90 sq. ft. which might require the removal of another cot. These bed closures occasionally caused local problems.

### Dieticians

They were considerably involved in translating the nutritional nedds of each child into an acceptable and palatable regimen (page 86 ).

### Physiotherapy, occupational therapy and radiography

These departments were involved but their work only slightly affected by the isolator. The isolator was as acceptable to them as to the nursing staff and this is discussed on page 79 .

### Electricians

A 20 amp power supply wired to the emergency generator was required. Fitting of five electrical plugs to flexes, earthing the supply and main isolator frameworks and provision of a plug-board on each supply isolator was their responsibility. All the electrical equipment was routinely checked after each patient.

### Fire and safety officers

The risks were probably no greater than for other patients but no electrical apparatus was used inside the isolator when additional oxygen was given. All electrical switches were outside the isolator. Short circuits due to spilt water twice occurred but only in the supply isolator as no electrical supply is present in the patient envelope. Low voltage circuits were not used (except for the bell call).

Building department

No capital building was required, and no ward alterations were made except as part of a minor programme of improvements. The isolators were assembled by medical and nursing staff, parents and medical students.

Porters

They were required to remove two beds and replace them later. They also arranged the weekly transport of materials for irradiation and the distribution of stores forwarded by the supplies department.

Administration

They were involved in press enquiries but this was not a specific effect of the isolators.

The nursing evidence showed that the Vickers-Trexler isolator could be integrated into a general paediatric ward and the child requiring a bone marrow transplant could be nursed within this environment by nurses from the ward establishment who also had responsibilities for nearby general paediatric patients. The hospital services were not affected in any major way by the use of the isolator particularly since almost all the additional supplies and stores required were a function of the marrow graft procedure and not items specifically necessary for the isolator. The isolator therefore fulfilled the aim of being a practical form of isolation which required no structural alterations to the hospital. In that it could be dismantled and removed to storage in about 6 working hours, or re-erected in another part of the hospital, the isolator gave a flexibility of bed space use not available in permanent classically-built isolation units.

Establishment and maintenance of the protected area

If the procedures required to obtain or to maintain an adequately protected environment are too difficult, complex or time consuming, there is little chance of the adequacy of the environment being maintained. Initial sterilisation of the isolator gave a sterile environment for the patient to enter. The techniques for producing sterile supplies and entering them

into this protected environment (described in appendix I ) with the routine "housekeeping" of the environment constituted the means of maintaining this environment. Major additional efforts were required to produce adequately decontaminated food since hospital foods as served on the wards may have an unacceptable bacterial content.

Sterilisation of the isolator

The filters were sterilised by 2.5 Megarads of gamma radiation and air at 50 cfm (1400 l/min ) passed through the filters for 5 minutes before connecting the filters to the isolator. This air flow gave a "blow-through" sterilisation of the input filter. Once the filters were connected the isolator itself was sterilised. The outlet filter, in communication with the isolator, received both the "blow-through" effect and the sterilising agent used on the inside of the isolator.

A 2% peracetic acid spray with 30 minutes exposure was recommended (Trexler 1978). This is highly effective against bacteria, viruses and fungi in addition to being sporocidal. Surfaces to be sterilised must be clean and in particular be free from grease. Peracetic acid vapour is as effective as the liquid preparation and so good penetration into awkward corners can be obtained. It has disadvantages in that peracetic acid must be made up less than four hours previously, has a very penetrating smell and is a co-carcinogen (Bock et al 1975). The undiluted product is also extremely toxic and needs care in its place of storage.

Although we used peracetic acid initially, alternative methods were sought. Gamma-radiation to 2.5 Megarads caused discolouration of the PVC after a second passage. Milton (sodium hypochlorite 1%) caused discolouration of the PVC after 3-4 hours immersion, presumably due to the hypochlorite radicals. This effect was lessened if the PVC was washed after being removed from the Milton. As 30 minutes immersion of non-metallic items in Milton is satisfactory for sterilisation; we determined whether a 30 minute soaking with Milton followed by washing with sterile water could replace peracetic acid as a practical method of sterilising the isolator envelopes. The use of either peracetic acid or Milton required the inside of the isolator

to be substantively dried after spraying.

Swabs were dipped in sterile saline and cultures taken from eight different sites twelve to fifteen hours after spraying and drying the inside of the isolator. The person taking the swabs had not sprayed that part of the isolator and deliberately sampled relatively inaccessible sites. The swabs were inoculated onto blood agar and cultured for 48 hours at 37°C. The results were as follows:-

COMPARISON OF 2% PERACETIC ACID WITH 1% SODIUM HYPOCHLORITE IN INITIAL STERILISATION OF ISOLATORS

| *After spraying isolator with | No. of studies | Total no. of sites sampled | No. of sterile cultures |
|---|---|---|---|
| 500 mls 2% peracetic acid | 10 | 80 | 80 (100%) |
| 750 mls 1% sodium hypochlorite followed by 750 mls sterile water | 6 | 48 | 48 (100%) |

*Half these volumes were used in the cot isolator

These results indicated that spraying with Milton, waiting 30 minutes and then washing with sterile water was an effective way of rendering the isolator sterile. Milton was henceforth adopted despite the extra labour of having to spray the isolator twice, once with Milton and once with sterile water.

Routine servicing of the isolator

The PVC envelopes were cleaned inside and outside each day and the entry and exit ports were cleaned before and after each use. The gloves and seams were checked visually every day. Punctures were repaired with sterile yellow PVC tape on the inside of the isolator, or resealed with 'PVX resealant' glue. The tightness of the triclamps was checked occasionally. External rough filters were washed or replaced every 2-3 months and the internal filters on the half-suit blowers cleaned every six months. The rough filter protecting the outlet filter was changed twice a week. The blower motors required no maintenance. The other electrical equipment was serviced according to standard hospital policies. The envelopes were replaced after about 250 days of use as were the main HEPA filters although no specific efficiency tests were carried out.

Apart from daily cleaning which would be required in any isolation facility there was therefore a minimal amount of routine servicing required to maintain the adequacy of the environment. Therefore establishment of the sterile environment and its daily maintenance did not constitute a major problem and confirmed the isolators to be a practical system. The question of their efficacy was however paramount. The intention was to provide an effective isolation system and a number of studies comparing the environment within the occupied isolators with their surrounding environment were carried out.

Microbial studies in the isolators

Studies were made of air within the isolators to determine the number of colony forming units (CFU) circulating in the isolator at rest and during activity. These results were compared with similar studies in the same ward, in cubicles and in a connecting corridor.

Air sampling studies

Studies were performed using an STA biological air-sampler which sampled 5 cubic feet (141 L) in 5 minutes through a slit onto a rotating blood agar plate. All air samples were obtained through a .78 inch (2 cm) diameter plastic tube 39 inches (1 m) long. This tubing was necessary to aspirate air from inside the isolator.

1. To ensure that the tubing used for air aspiration did not trap bacteria and give falsely low bacterial counts, eight parallel studies were performed in a corridor beside a staff changing room. In half the air entered the sampler inlet directly and in half the tubing was attached to the sampling inlet and the open end of the tubing held beside the sampler inlet and pointing in the same direction as it. The blood-agar plates were incubated at $37^{o}C$ for 24 hours and the number of colonies counted by the staff of the microbiology department. All results are quoted in colony forming units (CFU) per cubic foot (28 L) of air sampled. The results are shown in table 14a .

The number of organisms isolated shows a mean of 28.9 (range 12-37) organisms per cu. foot of air sampled. However there was no difference ascribed to the use of the air sampling

tubing and in particular there was no evidence that numbers of organisms were being trapped by this tubing and consequently failing to reach the culture plates.

2. A further study was made of four plates from each group to determine whether any group of organisms were being preferentially trapped by the tubing. The results are shown in table 14b, and although about 7% of the organisms were not immediately identifiable, there was no real difference between the major groups of identified organisms detected.

3. Studies compared the airborne bacteria in different locations. The sunny open ward containing the isolator, a corridor leading to the ward, cubicles where patients were being nursed normally or without protective isolation, were all compared to the air in the occupied isolators (table 15a).

The number of observations in the cubicles and corridors was somewhat small to give firm conclusions but the results lay between the ward and the isolator results. The isolator air had one tenth the density of airborne organisms compared to the sunny ward containing the isolator. Activity in either setting increased the airborne load two and a half times. Sixty one percent of isolator air samples were sterile compared to none in any other setting (table 15b).

4. A number of slit-sample studies reported the number of colonies of staph. aureus in different environments.

INCIDENCE OF STAPH. AUREUS IN AIR SAMPLES

| | Number of studies | No. of CFU | No. of colonies of staph. aureus | |
|---|---|---|---|---|
| Cubicle | 13 | 101 | 22 | 21% |
| Ward | 22 | 940 | 198 | 21% |
| Isolator | 77 | 336 | 0 | 0% |

On no occasion was staph. aureus isolated in air samples taken from the isolators whereas 21% of the CFU found in the open ward or the cubicles were staph. aureus. The proportion of airborne staph. aureus was the same in the ward, in the cubicles with reverse barrier nursing and in cubicles without any protective measures, thereby confirming the inefficiency of our cubicles as an effective barrier even in the presence of

Table 14a

ASPIRATING SYSTEM AND TRAPPING OF BACTERIA

| Aspirating system | No. of Studies | Total no. of CFU | Av. no. of CFU/plate | Av. no. of CFU/cu foot sampled |
|---|---|---|---|---|
| with tubing | 8 | 1217 | 152 | 30.4 |
| without tubing | 8 | 1095 | 137 | 27.4 |

Table 14b

ASPIRATING SYSTEM AND ORGANISMS CULTURED

| Aspirating system | No. of CFU | Staphs. | Aerobic spore bearers | Micrococci | Gram neg. bacilli | Others |
|---|---|---|---|---|---|---|
| with tubing | 380 | 130 (34%) | 89 (23%) | 100 (26%) | 2 | 59 (15%) |
| without tubing | 411 | 120 (29%) | 105 (26%) | 112 (27%) | 3 | 71 (17%) |

Table 15a

BACTERIAL CFU/CU. FOOT OF AIR IN DIFFERENT LOCATIONS

| Site | Activity | No. of Studies | Total No. of CFU | CFU/ cu. foot |
|---|---|---|---|---|
| WARD | quiet | 16 | 371 | 4.64 |
| | busy | 27 | 1671 | 12.38 |
| CORRIDOR | quiet | 4 | 36 | 1.78 |
| CUBICLE (normal) | quiet | 8 | 130 | 3.25 |
| | busy | 3 | 56 | 3.73 |
| CUBICLE (PE) | quiet | 5 | 40 | 1.6 |
| | busy | 3 | 22 | 1.46 |
| ISOLATOR | quiet | 35 | 75 | .43 |
| | busy | 54 | 278 | 1.03 |

PE - protective isolation

Table 15b

NO. OF STERILE AIR SAMPLES

| Site | No. of studies | No. sterile (%) |
|---|---|---|
| ward | 42 | 0 |
| corridor | 4 | 0 |
| cubicle | 19 | 0 |
| isolator | | |
| overall | 89 | 54 (61%) |
| quiet | 62 | 36 (58%) |
| busy | 27 | 18 (67%) |

as stringent protective measures as could be easily employed.

Other cultures

On only three occasions covering over 2000 microbiological cultures and 1217 days of use was a species of organism found on the patient or in the isolator which was not known to be present on the patient before entering the isolator. One organism, strep. viridans, entered through a tear in a half-suit visor and infected the patient. Large numbers of this organism were grown from the mother's eyebrows and nose. The other two organisms, both acinetobacter spp., were found on single occasions in a corner of different isolators and during at least 40 subsequent days of isolation, this organism was not recultured. However, as no phage typing or biotyping studies were carried out on routine isolates, additional contaminants cannot be entirely excluded.

Discussion

Table 16 shows the numbers of organisms isolated per 100 cu. feet of air sampled in different isolation facilities. My results of 43 and 103 colony forming units when the isolator was respectively quiet or busy compare well with those of Solberg et al (1971), Schimpff et al (1978) and Levine et al (1973), but are less good than those of Lowbury et al (1971) whose PVC isolator was unoccupied at the time of testing or Yates and Holland (1973) whose facilities may have been unoccupied when tested by air sampling. Bodey and Johnson (1971) had good results but their Life-Island air samples taken over eight hours at the same sampling volume per minute as my studies, were taken "through a port in the head board". The filtered air usually enters at the head end of the Life-Island so it is possible that much of each sample was freshly filtered air which had just entered the Life-Island. Most of my samples were taken at the "downstream" end of the isolator.

Not many studies quote the combination of air sampling with different degrees of activity within the protected area but in general activity greatly increased the airborne organisms though by much more than the factor of 2.5 in my study.

The results show that more sterile cultures were obtained

Table 16

AIR SAMPLING STUDIES IN DIFFERENT PROTECTED ENVIRONMENTS

Laminar Air Flow

| Author | Degree of activity | colony forming units/100 cu. feet of air sampled | % of sterile samples | organisms/cu foot in isolation compared to conventional ward (%) |
|---|---|---|---|---|
| Solberg et al 1971 | busy | 100 | NR | 0.3% |
| Bodey and Johnson 1971 | mixed | 0.5 | 72% | 0.2% |
| Levine et al 1973 | busy | 210 | 70% | 2.1% |
| Yates and Holland 1973 | ?unoccupied | 2.5 | 67% | 0.6% |
| Schimpff et al 1978 | ? | 100 | 56% | 37% |

Life-Island or PVC Isolator

| | | | | |
|---|---|---|---|---|
| Bodey and Johnson 1971 | mixed | 3.1 | 22% | 1% |
| Lowbury et al 1971 | unoccupied | <1 | NR | 0.1% |
| Levine et al 1973 | busy | 540 | 11% | 54% |
| Yates and Holland 1973 | ?unoccupied | 92 | 44% | 21% |
| This report Watson | quiet<br>busy | 43<br>103 | 58%<br>67% | 9.3%<br>8.3% |

NR - not reported

when my isolator was busy, but this difference was insignificant. The actual percentages of sterile air samples when my isolator was busy are very much the same as those recorded in the laminar air flow rooms of Bodey and Johnson (1971), Levine et al (1973) and Yates and Holland (1973) and Schimpff et al (1978), and are better than the results obtained by those workers using Life-Islands (table 16 ).

It is disappointing that there was only a ten-fold reduction in the number of airborne bacteria in the isolator compared to the sunny open ward. However Lowbury et al (1971) only showed such a ten-fold improvement when little ward activity was occurring around their unoccupied isolator. Other studies show reductions to between 1% (Bodey and Johnson 1971) and 54% of the ward airborne bacterial density (Levine et al 1973) when using slit sampling as the method of assessment. Results from units using laminar air flow units achieved greater reductions in airborne bacteria ranging from one fiftieth (Levine et al 1973) to one-fivehundredth (Bodey and Johnson 1971). The small reduction (one-third), quoted by Schimpff et al (1978) results from the low ward background contamination as their whole hospital was HEPA filtered. However the volumes of air required to maintain laminar air flow are greater than those required for a closed isolator. Schimpff et al (1978) used approximately 80 air changes per hour and Solberg et al (1971) used between 100 and 400 air changes per hour. The Vickers-Trexler isolator uses only 7 air changes per hour and therefore the load of organisms per total amount of air is very much less in the Vickers-Trexler isolator compared to the laminar flow units quoted in table 16.

The practical microbiological evidence is clear. The isolator provided an effective controlled environment with one episode in over 1200 days of use where an organism was known to have gained access, and that was due to a technical failure. Although the number of airborne organisms was only a tenth that of the surrounding ward at no time was staph. aureus found in air samples from the isolator whereas it was cultured from 21% of the colony forming units found in the air of both the ward and the cubicles, including those which were supposedly providing protective isolation.

Acceptability of the isolators

The evidence shows that the isolators have provided an effective form of isolation as used within a general paediatric ward. The question of just how acceptable the isolators were to the children, their relatives and to the staff is equally important. Two persons out of over 200 found the half-suits intolerably claustophobic but these were the only persons to whom the isolators were quite unacceptable.

Temperature

The isolator was usually in a large open sunny ward. With thermometers inside and outside the isolator, numerous recordings at 0900, 1200 and 1800 hours were obtained. The temperature in the isolator was occasionally 1C$^{o}$ warmer than the ambient ward temperature. However when direct sunlight struck the isolator, a "greenhouse" effect resulted in an increment of up to 5C$^{o}$. This effect was eliminated by drawing the appropriate blinds. Attempts to air-condition the isolator failed because the large surface area gave so much heat exchange. The only solution would be to air-condition the whole ward.

Noise levels

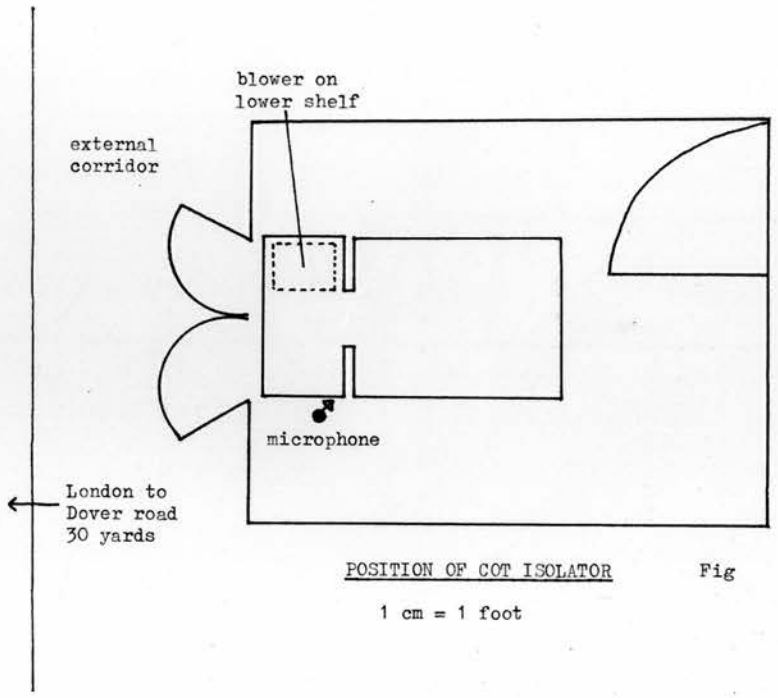
Studies were made on a cot isolator which had a 20 cubic foot per minute (cfm) blower and on the bed isolator with a 50 cfm blower and a 5 cfm half-suit air supply blower. These were compared with a neonatal intensive care incubator and a Humidaire tent in a ward.

Cot isolator studies (figs. 4 and 5)

Noise levels were recorded using the dbA scale two feet (61 cms) into the cubicle containing the isolator with the windows of the glass balcony closed. The microphone faced into the cubicle which was 30 yards (27m) from the London to Dover road. The 20 cfm isolator blower was 2 feet 6 inches (76 cms) from the microphone. In figure 5, the background lay between 49 and 57 decibels (db) depending on passing traffic. A London Transport

# NOISE RECORDING STUDIES WITH THE COT ISOLATOR

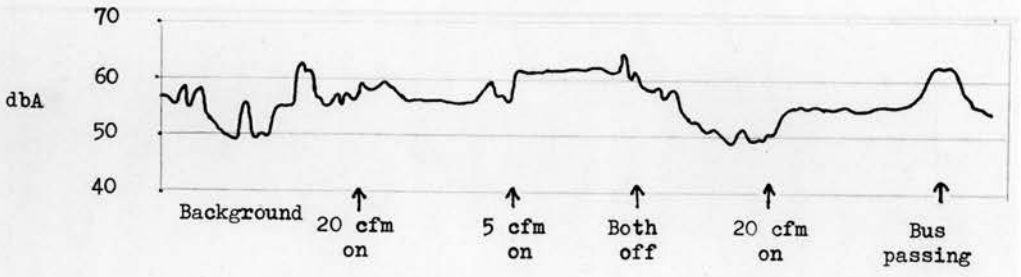
## Position of recording microphone (figure 4)




POSITION OF COT ISOLATOR Fig

1 cm = 1 foot

## Noise levels recorded (figure 5 )




NOISE LEVELS RECORDED OUTSIDE COT ISOLATOR Fig

(transcription)

bus passing 30 yards away gave a reading of 65 db; an increment greater than that of the 20 cfm blower. Isolator induced additional noise to which staff outside this isolator were subjected was less than that produced by variations in the normal background.

Bed isolator studies (figs. 6 to 9 )

Figure 7 shows the contribution of an isolator to the noise in a general ward which faced a quiet square in central London. There was no difference in recorded noise whether the microphone faced the isolator or faced into the ward, the general level of ward noise lying between 55 and 65 db. During the children's afternoon sleep the level fell to 44-52 db. At tea time the noise frequently exceeded 65 db. The main 50 cfm blower did not apparently add to this but the 5 cfm half-suit blower, without air waistcoats attached, raised the noise level to a consistent 63 db. Re-attachment of the air waistcoats halved the increment, and gave the normal working circumstance, a noise level of 57 db. It was to this that staff immediately outside this isolator were exposed, a similar figure to the ward background noise.

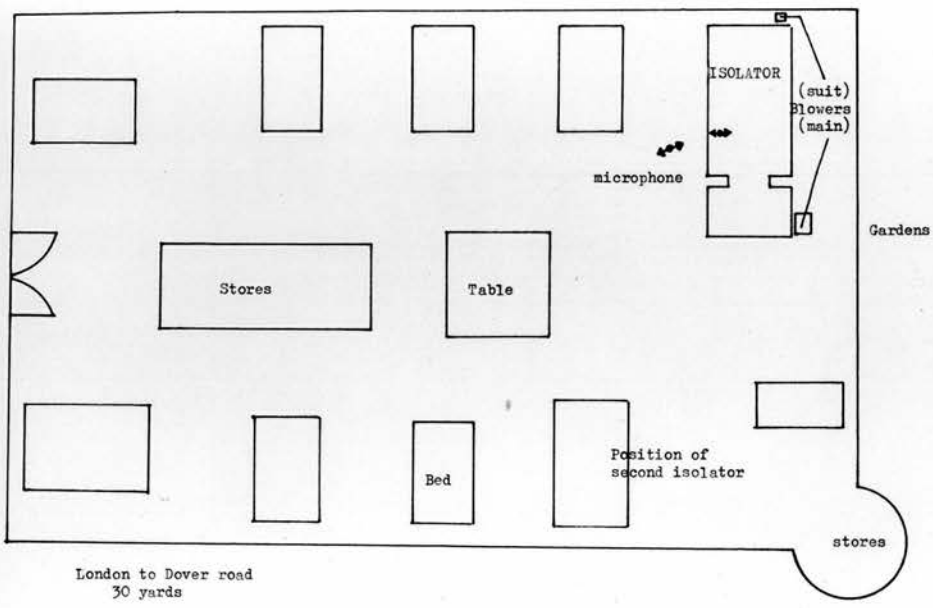
Noise levels inside the bed isolator were compared with the noise levels outside the isolator (fig. 8 ). This shows the ward background to be 48-58 db whereas in the isolator it was a few db less. With both blowers running the level was 60 db. This is slightly more than that shown in figure 7 where the steady level outside the isolator was 57 db, probably due to the microphone being slightly nearer the 5 cfm blower. It did mean that the child was subjected to 55 db continuously and 60 db when attendants forgot to switch off the half-suit blower. Meals in the ward often exceeded 75-80 db as recorded at the isolator and the effect of the blowers was noticeable only on recording where they raised the lower noise levels from 50 db to 58 db.

Staff working in a half-suit experienced considerable noise levels (fig. 9 ). From a background of 50 db the air-waistcoat receiving 1.6 cfm raised the noise level in a half-suit to 66 db. For auscultation some staff switched off the half-suit air supply (bearable for 3-5 minutes).

Pitch recordings were also performed (not shown). Much of the noise from the 50 cfm blower was less than 100 cycles per

# NOISE RECORDING STUDIES WITH THE BED ISOLATOR

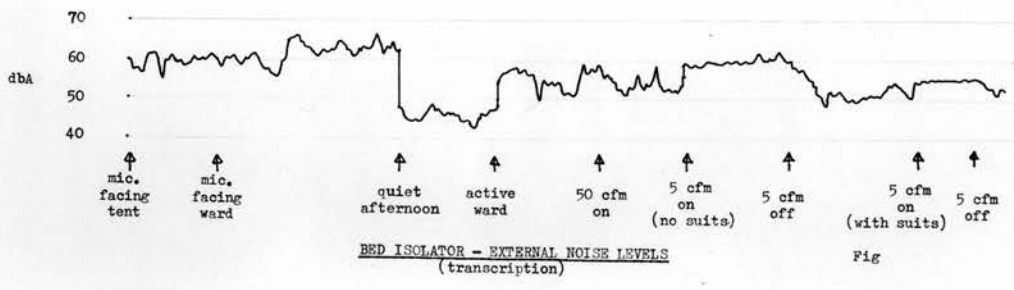
## Position of recording microphone (figure 6)




POSITION OF BED ISOLATOR Fig

1 cm = 2 feet

## Bed isolator noise levels (external) (figure 7)

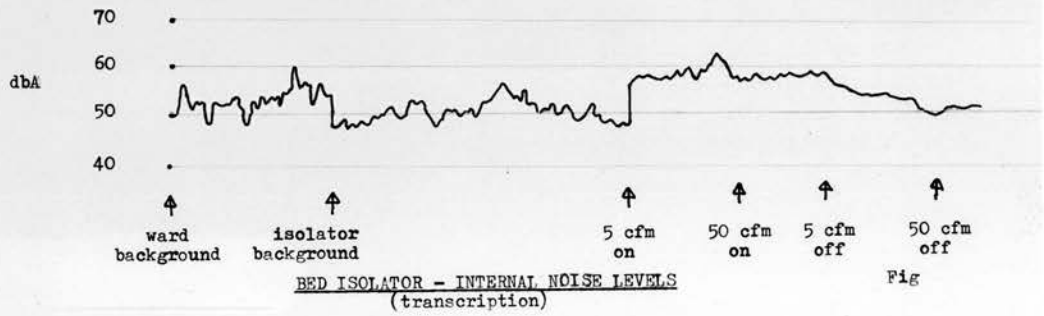



BED ISOLATOR - EXTERNAL NOISE LEVELS
(transcription)

Fig

## NOISE RECORDINGS WITH THE BED ISOLATOR

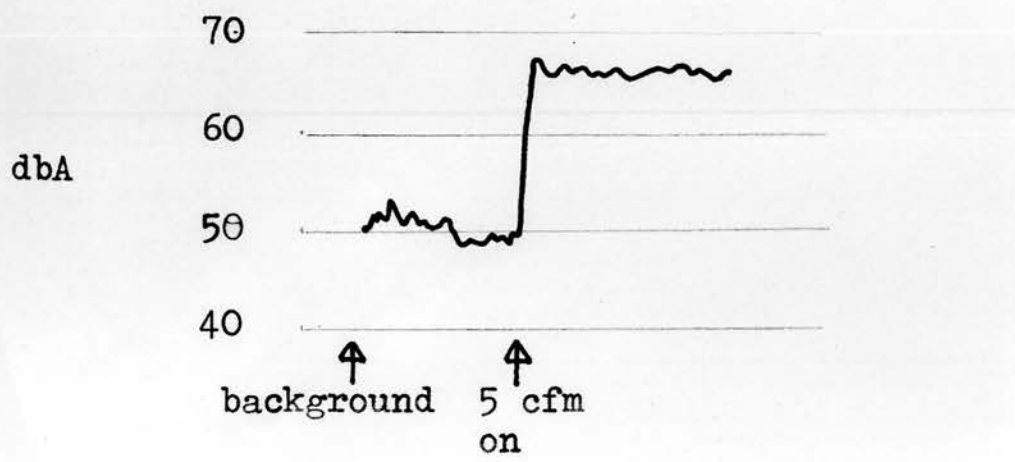
### Internal noise levels (figure 8)




BED ISOLATOR - INTERNAL NOISE LEVELS
(transcription)

Fig

### Noise experienced by staff in a half-suit (figure 9)

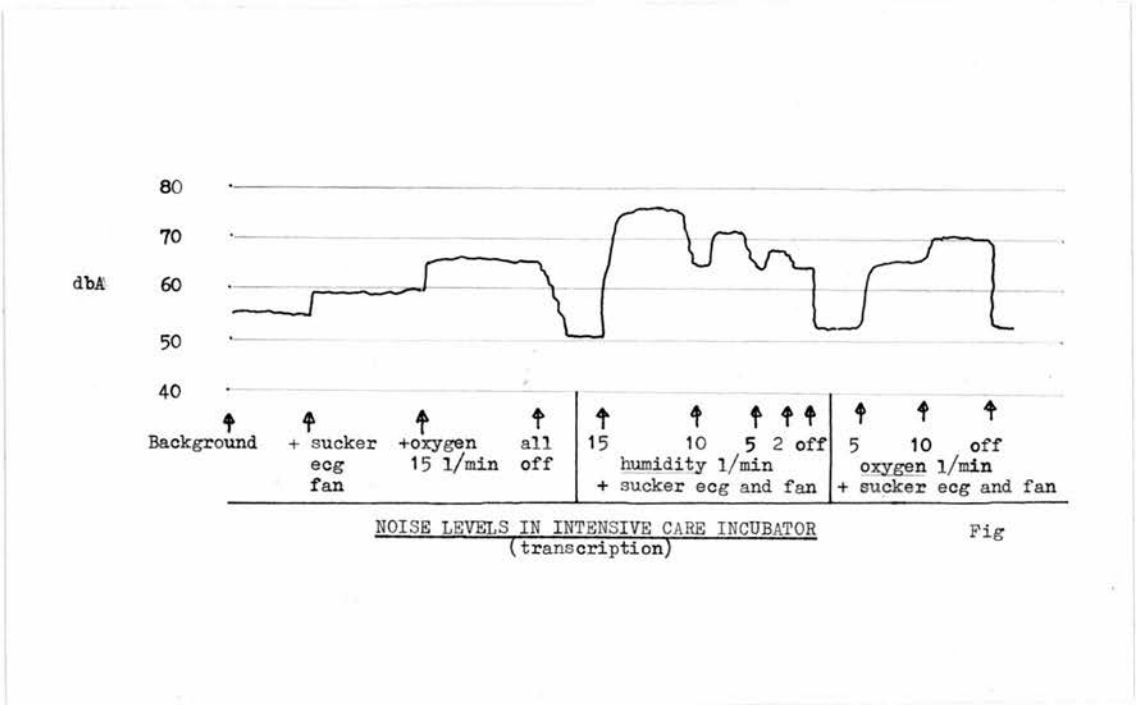



NOISE LEVEL WITHIN A HALF-SUIT
(transcription)

Fig

second (cps) and very little was above 1600 cps. Similar studies were performed on other hospital equipment. Fig. 10 shows the noise level in a Vickers Medical 59 incubator with additional equipment in use. Extra humidity was supplied with an air flow of 2-15 L/min. At 5 L/min the infant was subjected to a constant 70 db. This level of noise has been described by Harris et al (1978). Similar studies in a Humidaire tent showed that a child received a constant 64 db.

NOISE RECORDING IN AN INCUBATOR (figure 10)




NOISE LEVELS IN INTENSIVE CARE INCUBATOR (transcription) Fig

Combining the results of these assessments of the noise generated by the bed isolator showed that the mean background noise beside the isolator was 56 db (range 48-64 db). The 50 cfm blower and 5 cfm blower in normal operation gave a mean operational level of 58 db. Background levels were 2-3 db lower in the isolator and noise levels when in operation were 1-2 db higher than the external recordings. Figures for the cot isolator were similar with the infant subjected to about 58 db. Simple hearing tests have been normal, and on infants in isolators for several hundred days apparently normal development of hearing responses occurred. Apart from when staff used stethoscopes in the isolator, no problems associated with the noise of the blowers was experienced.

Acceptability to patients

If at all possible the children were shown the isolator or else large photographs of the system before admission. The parents and child could try the half-suits but unless they so asked they did not get inside the isolator. Similarly they did not try the sterile foods in case such experiences produced a negative reaction for whatever spoken or unspoken reason with an increase in anxiety for all around. Methods of sterilising toys, clothes and their personal possessions were discussed to give as full a range of entertainments and diversions as possible. Patients who had previously been in the isolator undergoing bone marrow grafting, or their parents, were very helpful in explaining many aspects of the whole experience to other families.

Children adapted much better to the isolators within a general ward than did adults (Watson et al 1977). Without doubt, their physical condition was the greatest determinant of their psychological well-being. Initially the children found the isolator entertaining and they variously regarded their isolator as a castle, a camping tent or a 'Wendy House', and the attendants in the half-suits as space men or ' Wonder Woman'. These first reactions rapidly changed and after a week they accepted the isolator for what it was and became efficient in managing their environment. Over the next weeks they became critical of staff techniques and knew as much of the procedures as the nurses. A new nurse starting at this time might feel threatened by her patients' knowledge and her own lack of knowledge. This needed tactful handling as the parents or child could reject an uncertain member of staff, partly as an expression of their own anxieties.

After four weeks, the children became ambivalent towards most situations, with their reactions swinging markedly from hour to hour. At times they became very withdrawn, especially if unwell, hiding under the sheets and rejecting their drugs and visitors. This reaction was initially interpreted as boredom, but was really a product of apparently endless time, investigations and negative feelings about the future. Emotions became very labile with often insistence on one particular staff member to do certain tasks and rejection of all other help. These critical and rejecting episodes were difficult for parents to

accept as they were not part of the customary family behaviour, and some parents felt their child was "letting them down". Threats to leave isolation were common but never enacted.

After about 6 weeks the child's physical condition determined their most frequent behaviour pattern. Most of the pre-teenagers who were well, realised that only a short further time was needed in the isolator and frequently "played to the gallery", thoroughly enjoying the attention. Before leaving the isolator feelings of insecurity were occasionally manifest and returning to an environment where they were no longer the VIP gave occasional problems. After discharge the children were somewhat self-centred and some had difficulty relating with school friends. The desire for tactile stimuli was noticeable for some days; attention span was reduced and verbal activity increased. Over six months these all returned to normal. Recurrent dreams of the isolation period only once occurred.

It was important to organise parts of the child's day in the isolator. A quiet rest period had to be provided when even staff were discouraged from disturbing the child. Time set aside for the school teacher indicated that the future existed to both parents and child. Although visits from pop stars or footballers were greatly appreciated also by other patients in the ward, too many inquisitive visitors made the children feel they were in a "goldfish bowl". Contact with other patients was encouraged and usually enjoyed. With the isolator in a general ward, participation with the ward school teacher and entertainments was possible. The isolator did not prevent the occupant shouting to his friends in the farthest corner of the ward. Curtains and a folding partition allowed the isolator to be screened off from the ward if necessary.

Should death be approaching, there was the choice of leaving the child in the isolator or removing him to allow close family contact. Some parents felt that removal from the isolator would indicate to the child (and themselves) that hope was gone. Others regretted that they did not have close contact with their child at death. There was no specific policy except to respect the parents wishes. On balance I would prefer to move the child to a cubicle.

Acceptability to relatives

With the isolator and therefore their child being in a general ward was helpful as the parents developed relationships with other parents and other children. Since the mean stay in the ward isolator was 65 days, parents often developed a surrogate role with another child. This role, in addition to physically helping with the nursing of their own child, allowed displacement of some tension and anxiety and meant that the parents were not totally displaced from their traditional role. The isolator techniques were easily taught and parental help was actively encouraged.

Visiting the child was greatly eased by the minimal training necessary compared with the training required to maintain isolation successfully in a cubicle with full aseptic precautions. The technique has to be correct every time a cubicle is entered whereas the isolation is never broken by a visitor to the isolator tent. Brothers or sisters with a respiratory infection could just as readily visit the patient, whereas this could not be allowed in a cubicle. They would not however enter a half-suit as the staff would then be at some increased risk of infection. A parent could sit beside the isolator or in a half-suit, just to be with their child and knit or read whereas this might be less acceptable in cubicle protective isolation.

A new toy or electronic television game could be immediately used with the child in the isolator operating it through one of the sleeves in competition with his visiting sibling outside. The controls of a television set were equally accessible, and no extra audic equipment was required. Letters and postcards were read through the PVC envelope and then sterilised and passed into the isolator or sellotaped to the envelope.

Acceptability to staff

The major benefit was the ease of access to the patient compared to a standard hospital cubicle. Casual conversational visits, and quick reviews of certain problems were much more easily performed than when the child was in a cubicle with full reverse barrier precautions. Routine nursing observations were made without entering a half-suit by a nurse who could look

after other patients in the same ward. Rotating intravenous feeding regimens and intravenous drugs were given through the intravenous line outside the isolator; thus two nurses did not have to gown up, and senior staff checking intravenous drugs were not hampered in their other work.

Nurses were not confined for considerable periods of time with the children, which in protective cubicle nursing leads to progressive inefficiency of protective clothing. The presence of less critically ill children around the isolator helped alleviate emotional strain for all members of staff. Occasionally both student and staff nurses found certain duty rotas arduous especially when the isolator patient was very seriously ill but this was not a problem confined to isolator or transplant patients. Changes in duty rotas and sympathetic handling of the situation forestalled some of these problems.

The nurses trusted the isolators, and felt that much of the responsibility for the prevention of cross-infection had been alleviated for them. The actual nursing was little different in the isolator, although some improvisation might be required. This did not imply any reduction in the standard of nursing but more a planned approach to each action. The senior ward staff found the discipline, planning and responsibility required in isolator nursing helped some student nurses become more methodical and foresighted about their work. Such special emergencies as might arise with the isolator itself, e.g. loss of inflation pressure, had procedures to be followed but this was a matter of prior education for both nursing and medical staff.

The medical staff concurred with the nurses' opinions and saw the same benefits as the nurses for the patient and themselves. The reliability of the isolator was a considerable benefit and the need for no protective clothing saved time. Several staff members present at a consultation could all examine the patient without numbers of people breaking isolation by entering a cubicle. Discussion needed the same sensibilities as with any patient since sound transmission was good. Casual social visits were very easy and talking to parents did not require them to leave a cubicle and regown afterwards.

Medical procedures were performed in the isolator with a minimum of difficulty. The thickness of the gloves reduced sensation a little, but venepuncture could still be performed solely by palpation. Surgical procedures such as opening of abscesses, insertion of long intravenous lines via the internal jugular or subclavian veins were all performed. Gloves were changed aseptically to accommodate the surgeon's requirements. Investigations such as a barium meal, electroencephalography electrocardiography and ultrasound studies were all carried out without breaking the sterile barrier. Stethoscopes with a paediatric head were passed down a sleeve into a glove (with or without a hand in the glove) and easily used, though the benefits of the bell were lost. Chemical sterilisation of an ophthalmoscope would ruin the lenses, but those who learned the knack could use the instrument from outside through a half-suit visor. Major resuscitation is still not possible in the isolator, but common sense dictated the immediate policy. Specific instructions were always in force and reviewed frequently.

There was no doubt that the isolator was acceptable to the relatives and the members of staff. The access to facilities and contact with other patients and staff made it the least isolating form of isolation available for children of all ages and they accepted it well. Whilst similar arguments apply to laminar air flow units regarding the degree of isolation from the social environment, the lack of necessity for any protective clothing was a major advantage for the staff and relatives.

## Discussion

This is an extension of the only reported evaluation of isolators for bone marrow transplantation on an open ward (Watson et al 1977). It is also the only such evaluation involving solely children being cared for in a general ward of a general children's hospital and not in a special anaemia, leukaemia or transplant unit with its own specialised staff.

A comparison can be drawn between the isolator and other forms of protected environment (Table 17 ). The isolator in common with cubicles and LAF rooms provides a barrier which is independent of air flow. A LAF bed is totally dependent on the air flow to maintain the protected environment. Air filtered

Table 17

COMPARISON OF DIFFERENT ISOLATION FACILITIES

| | Hospital cubicle | Cubicles Purpose Built with filtered air | | LAF | LAF room | Isolator |
|---|---|---|---|---|---|---|
| Physical barrier | + | + | + | − | ± | + |
| Barrier dependent on air flow | − | − | − | + | ± | − |
| Protection against airborne organisms | − | − | + | + | + | + |
| Air-conditioning required | ± | ± | ± | − | − | − |
| Easy use of bed for other purposes | + | + | + | + | + | ± |
| Easily removed and reassembled elsewhere | − | − | − | + | ± | + |
| Building modifications required | − | + | + | − | ± | − |
| Easily kept clean | − | + | + | + | + | + |
| Barrier breached to care for patient | + | + | + | + | ± | − |
| Protective clothing required by staff | + | + | + | + | ± | − |
| Very rapid access in emergency | + | + | + | + | + | ± |
| Good visibility of patient | ± | ± | ± | + | ± | + |
| Considerable social contact | − | − | − | + | + | + |
| Protection from careless visitors | + | + | + | − | ± | + |

to HEPA standards is often difficult to fit to a normal hospital cubicle but is available giving both protection against and dilution of air-borne organisms in specially constructed cubicles, LAF units or isolators. Additional air conditioning is not required for LAF units or the isolator but may be necessary in cubicles depending on their nature. If not required for their primary purpose, the beds in cubicles or LAF can be readily used for other purposes, but should the isolation facility not be required for some reason, only LAF units and isolators can be removed and the space put to other uses. LAF and isolators can be re-assembled in other sites and no building modifications are required when using isolators or LAF.

Normal hospital cubicles are difficult to keep clean unlike units specially designed for protective isolation. Caring for the patient requires the barrier to be breached in all systems except the isolator and the LAF room where some procedures may be performed through the wall of the room.

Staff attending to or examining the patient must take full aseptic precautions every time in all systems except the LAF room and the isolator. Sudden emergency access to the patient is not quite so easy in the isolator as in other systems since a generous slit must be made in the PVC but the ability of staff outside the isolator to see the patient is considerably better that when the patient is in a cubicle. LAF and isolators give the patient much more contact with other patients and visitors than any cubicle system but the physical barrier of the isolator or LAF room prevents the careless visitor from breaking the protective barrier

The isolators therefore provide an effective practical protected environment which has a number of advantages over other systems, particularly of the cubicle design. There are fewer advantages over LAF but the major ones remain of a physical barrier which is not broken by the attending staff who therefore need no protective clothing, and only require to wash their hands before attending to the patient. The objective in using isolators was to provide practical effective isolation within a seventy year old children's hospital which had not undergone major modernisation and would not be able to find the construction or staffing of a designated unit. This objective was achieved.

Use of an exclusion isolator for containment

An additional use of the isolator presented itself when episodes of cross-infection occurred within the infant ward. When used with positive pressure, the isolators exclude airborne infection whereas with internal negative pressure, infection within the isolator may be contained, protecting the attendants from air disseminated organisms. Although the PVC envelope prevents contact transmission, care is still required in the disposal of infected material. These techniques with a containment isolator were described by Trexler et al (1977). Such containment isolators are available at designated hospitals in the United Kingdom for patients with dangerous fevers, e.g. Lassa or Marburg fever. These isolators have the same basic design as those used for exclusion of micro-organisms at Westminster Children's Hospital and all modifications made to the basic design must maintain industrial compatibility even though the ultimate use of each isolator is different.

No opportunity had arisen for an isolator usually used for exclusion isolation to be converted and used for containment isolation in the same institution by staff familiar with nursing in the exclusion mode. We had thought that such conversion and management could be achieved and put this into practice when presented with an appropriate situation.

Case report

D.E., aged 3 months, weighing 2.6 kg presented from the Middle East with severe diarrhoea for 2 months. On initial investigation, Salmonella wein was isolated from the stools on several occasions and ultimately infection by this organism was the final diagnosis. The child was fed intravenously for 97 days and very slowly regraded to normal feeds via a diet of comminuted chicken with mineral, lipid and carbohydrate supplements. Cubicle isolation and barrier nursing was effective until 128 days after admission when three episodes of cross-infection amongst other infants occurred. On each occasion a different nurse was found to excrete S. wein in her stools. Despite rigorous protective measures with five nurses being allocated to look after only this child and a short course of antibiotics to

the index case, a further infant became colonised. Within a cubicle the infant was placed in a Trexler cot isolator converted from exclusion to containment mode. No further cross infection or positive environmental cultures occurred during the 113 days she spent in the isolator. On discharge home, weighing 8.3 kgs, Salmonella wein was still present in her stools.

Technical alterations

A standard cot isolator with supply envelope and framework was converted to the containment mode. Since the patient envelope of an exclusion mode cot isolator has its structure maintained by positive air pressure without any supporting framework, the use of negative pressure would have caused the envelope to collapse around the child. The envelope was therefore kept in position by stretching Vickers "Oxymist" tent elastic cords along the length of the cot and using the "Oxymist" pegs to secure the patient envelope to the cords. This gave adequate support. The PVC envelope was protected with PVC adhesive tape applied where the pegs were positioned. The supply isolator had its own framework so no additional support was required.

The air supply required alteration. In the cot isolator filtered air enters the patient envelope via a sleeve and diffuses out through the entry port. This could not have been satisfactorily reversed so a new port was cut in the roof of the supply canopy and through a 6" (15.25 cm) HEPA filter, air was drawn out of the isolator to the suction side of the blower. The patency of the usual air entry sleeve was maintained with spacers and ward air entered this passively through an 8" (20 cm) HEPA filter. An additional similar sized passive air entry was found necessary to allow an adequate number of air changes per hour within the isolator.

A standard 14" (35.5 cm) diameter side entry port was used to enter and exit items from the isolator. The procedures were exactly as described for Lassa fever patients by Trexler et al (1977) and required Panduit ties and a Weller heat gun to separate and further seal discarded infectious packages. Gloves and glove rings were fitted from outside instead of from inside the isolator. These modifications took 4 hours and setting the isolator up took another hour. The ward nurses

were familiar with the techniques required for exclusion isolation of the marrow transplant patients. No particular nursing problems were encountered and the nurses accepted this form of source isolation extremely favourably (Fradd 1979). The great benefit to the ward and the nurses was that no longer did this child require nurses specifically for her. This meant that these nurses now worked throughout the ward though it did seem wise to exclude nurses working with the infant from the milk kitchen. The patient benefited in that no longer was she visited as infrequently as possible but people could now visit her socially.

Discussion

Such cross- infection as occurred when the child was nursed in the cubicle should not have happened and it could be argued that the infant should have been cared for not in a general paediatric environment, but in a proper isolation unit. Those locally available to us could not have adequately managed the intravenous feeding of an infant weighing 2.6 kg. The subsequent events show that infants can be readily cared for in containment isolation using flexible film isolators, and that an exclusion mode isolator can be readily converted to containment by non-industrial staff. This dual use preserves skills in the nurses who may not be continuously employed in exclusion isolation. A containment isolator in a general paediatric ward fulfilled its two objectives, protection of the staff and patients and freeing nurses for ward duties.

In addition, it was evident how much more social contact the infant received when she was in the isolator compared to when she was in a cubicle with source isolation procedures.

A PATHOGEN FREE FOOD SUPPLY

Introduction

The paediatric isolator formed an effective method of achieving isolation within a general ward and elaborate arrangements enabled a supply of sterile equipment to be always available. However a major problem lay in providing food of an equally high microbiological standard. Pathogenic bacteria frequently contaminate the conventional food offered to hospital patients but this, as a source of infection only becomes of importance when

other techniques have eliminated patient-to-patient or staff-to-patient cross-infection.

Shooter et al (1969) showed that 8% of food samples from their hospital kitchen contained pseudomonas spp. as did one-fifth of surface cultures taken from either the main kitchen or the diet kitchen. Although cultures from the main water tanks were sterile, the kitchen taps were frequently contaminated especially if a detergent dispenser was attached. Salads and cold foods were the foods most frequently contaminated with pseudomonads, esch. coli or kliebsiella spp. (Shooter et al 1971) but hot food was not entirely exempt. One-third of milk based feeds were similarly contaminated with intestinal organisms.

While much of this food contamination is at a low level, 2.5% of food samples contained more than $10^3$ organisms per gram (Shooter et al 1971). Although the number of esch. coli required to colonise a healthy patient is probably between $10^5$ and $10^6$, the number required to colonise an immunosuppressed patient who is taking oral prophylactic antimicrobial agents is not known. Wherever the actual source of the organisms, about 25% of patients in a general hospital ward for 3 weeks become colonised with new pseudomonas spp. (Shooter et al 1969) and some of these must be acquired via the normal hospital diet. This makes the use of some form of specially prepared food essential for patients undergoing bone marrow transplantation, especially as the intention is to prevent the patient being colonised by all exogenous gram negative organisms.

Although trials with neutropenic patients comparing a normal diet with a sterile diet as the only variable have not been reported, Priesler et al (1970) compared a "cooked" diet with a "sterile" diet during remission induction of adult AML. Of their 21 patients, those taking the sterile diet had a greater percentage of sterile stool cultures but showed no particular clinical benefit to result from the "sterile" diet. Vossen and Van der Waaj (1972) found all their samples of a steam-sterilised diet to be sterile compared with half the samples of a pasteurised diet and 8% of the samples of the pasteurised diet contained large numbers of bacteria. This source contributed considerable contamination to their ultra clean rooms.

Most decontamination regimens have included a special diet. Usually this excluded uncooked dairy products and fresh vegetables and was specially cooked in a separate kitchen with either an autoclave or steam-sterilisation facility. Careful transfer arrangements were required to take the prepared food to the patient who used autoclaved crockery and cutlery. Establishing a special kitchen and transfer arrangements requires both space and capital. My need was to establish a pathogen-free food supply for the children undergoing bone marrow transplantation. Whilst exclusion of certain dietary items would have helped, the problem remained of delivering pathogen-free food to children in the isolators within a general ward in a hospital which had no diet kitchen or cooking facilities outwith the normal hospital kitchen. The small ward kitchen (120 sq. feet) was already used for serving and washing-up the other children's meals and storing the ward crockery and cutlery. Therefore an alternative had to be found.

Methods

Because there was no real possibility of cooking elsewhere and transferring the cooked foods to the isolator patient, the nurses and children's parents cooked within the isolators. Each isolator contained a pop-up toaster, automatic electric kettle, mark 2 Sunbeam multicooker and a small refrigerator. Food passed into the isolator therefore had to be of an acceptable micro-biological content. Infants' feeds were made up in the isolator and a twelve hour supply stored in the refrigerator. For the children a more varied diet was required.

Commercially available good quality canned foods were accepted as pathogen-free providing the can was undamaged. The label and glue were removed, the can labelled with a felt-tip pen and the surface of the can sterilised in Milton for 30 minutes. The can then entered the isolator, was opened with a sterile can-opener and cooked. Autoclaved or Long-Life milk and sterile water was treated in the same way.

Individual packs of frozen food were delivered to the deep freeze. This clean food was sealed in two nylon film bags with the date and a radiosensitive marker on the inner bag and returned to the freezer. Subsequently the frozen non-sterile

packs received at least 2.5 megarads from a Cobalt-60 source and then returned to the hospital freezer. Bread, cereals, tea, sugar, coffee, salt and pepper were packed in convenient amounts and treated in a similar but non-frozen way, as were packs of disposable plates, bowls, cups and cutlery.

Supplemental feeding was also used, particularly when mouth ulcers or dysphagia was present. Sip feeds were sometimes successful as were fine-bore nasogastric silicone tubes but these were not very convenient and even when in the duodenum or jejunum were regurgitated and had to be repassed almost daily. Intravenous feeding was used for a time for almost all children. A simple intravenous supplementary regimen administered through the central venous line supplying 60 kalories/kg/day, with 2.1 gms of aminoacids/kg/day proved adequate. 30 mls/kg/day of vamin-glucose and 100 mls/kg/day of 10% glucose with additional electolytes and fluid supplied 60 kalories/kg/day without excessive glycosaemia. For infants this was calculated on expected rather than actual weight. This intravenous regimen was inadequate for growth but complemented the oral intake.

Discussion

The microbiological results of this food preparation policy were entirely satisfactory. From time to time specimens of freshly opened irradiated food or samples from freshly opened cans of food were cultured in broth and then on blood agar and Maconkey agar at 37°C for 24 hours. Of twenty such specimens, only one, a specimen of roast chicken, grew a few colonies of bacillus spp. and the relevance and true source of this remained in doubt. Foods were not sampled microbiologically before radiation since this would inevitably have been a random process and such an approach is not employed for public health surveillance purposes (Charles 1979). Having proper techniques of preparation of food is a more effective way of ensuring a high standard of food hygiene. Since our foods were derived from many sources, irradiation where possible was the most convenient policy.

Gamma irradiation to 2.5 Mrad is within the guidelines of the Ministry of Health Working Party on Irradiation of Food (1964) but is a compromise dose to reduce the microbiological content, and yet maintain palatability. Since irradiation does

not destroy autolytic enzymes, irradiated food remains perishable unless frozen at minus 20°C. Our food remained palatable for at least a year. The use of radiation increased the variety of foods considerably giving a choice (to be selected 2 hours before cooking time) of 33 main dishes, nine different vegetables and ten desserts (table 18 ). No new irradiated food was offered to a child until the staff first tasted a sample. Some problems encountered with this irradiation are summarised in table 19 . There were disadvantages in this diet, mainly due to the lack of fresh vegetables. Extra vitamins were given to all children either as Ketovite tablets and elixir, multivite tablets or multivitamin infusion with additional Parentrovite A and B. All children received at least one gram of vitamin C per day.

A fine-bore nasogastric tube had problems in addition to vomiting of the tube. Even without an oesophageal foreign body, oral candidiasis may spread to the oesophagus. In the presence of a nasogastric or nasoenteric tube, staff tended to give drugs via this route. If the oral antifungal agents were given thus, oral candidiasis became more prevalent. Oral hygiene was further compromised by the reduction in the mechanical effects of drinking and so greater attention to the oral cavity by the nurses is required when a gastric tube is in place.

Liberal use of nutritional supplements such as Build-up, Bengers, Caloreen and Hycal was made where possible. The most convenient fluid food was Isokal on account of its isotonicity, sterility and freedom from lactose. Since it is ready for use no preparation is required and it could drip slowly straight from the tin using the special adaptor. Infants with severe combined immune deficiency almost invariably had secondary lactose intolerance so received Galactomin 17, comminuted chicken, or Prosobee. Their oral drugs were also lactose-free.

The intravenous feeding policy varies in different centres. Most patients in the Seattle transplant unit receive intravenous feeding but it is only occasionally necessary at the Royal Marsden Hospital. Three-quarters of their transplant patients lose weight during their admission with a mean weight loss of 7%. Those aged 16 or less lose an average of 12.5% of their admission weight and the only patients of the most recent

Table 18

## STERILE FOODS AVAILABLE FOR MAIN MEALS

| Soups | Fruit | |
|---|---|---|
| Tomato | Fruit salad | Strawberries |
| Chicken | Apricots | Orange juice |
| Spring Vegetable | Pears | Pineapple juice |
| Oxtail | Peaches | Grapefruit juice |
| Beef Broth | Mandarins | Tomato juice |
| Mushroom | Raspberries | |
| **Main Dishes** | | |
| Macaroni cheese | Canneloni | Kidneys in gravy* |
| Ox tongue | Pork frankfurters | Liver and onions* |
| Corned beef | Spaghetti | Cod in butter* |
| Ham | Fish fingers* | Roast beef* |
| Salmon | Fish fillets* | Leg of pork* |
| Baked beans | Prawn curry | Shepherds pie* |
| Baconburgers | Beef and dumplings* | Roast chicken* |
| Chunky steak | Sausages and onion* | Beef and onions |
| Chunky chicken | Steak and kidney* | Pork paté |
| Minced beef | Chicken and mushrooms* | Lamb chops* |
| Ravioli | Lamb casserole* | Pork chops* |
| **Vegetables** | **Desserts** | |
| Peas | Sterilised cream | |
| Carrots | Rice pudding | |
| Potatoes | Custard | |
| Tomatoes | Chocolate dessert | |
| Potato salad | Strawberry dessert | |
| Cucumber salad | Treacle pudding | |
| Vegetable salad | Mixed fruit pudding | |
| Cauliflower* | Golden honey pudding | |
| Green beans* | Chocolate pudding | |
| Brussel sprouts* | Instant whips | |
| Smash* | Yoghurts* | |

*Gamma irradiated

Table 19

PROBLEMS ENCOUNTERED IN RADIATION OF FOOD

| | |
|---|---|
| Glass | goes black |
| Polo mints | go pink |
| Jelly babies<br>Blackcurrant syrup<br>Orange syrup | lose all colour<br>(but not taste) |
| Oranges<br>Pickled onions<br>Eggs | disintegrate |
| High carbohydrate drinks | caramelise giving off carbon dioxide which ruptures non-pressure bottle |
| Grain derived foods,<br>e.g. bread, cereals | taste if wrapped in plastic so use nylon film |
| Foods with more than 15% fat [1,2] | turn rancid |

[1] Many exceptions
e.g. peanuts (49% fat)
bacon
crisps
mince
pork
steak and kidney pie
bourton cream biscuits

[2] some foods with less than 15% fat do not irradiate acceptably
e.g. croquette potatoes
suet dumplings
chocolate (including Build-up, Instant Whip)
reddi-brek (8.7% fat)
bournvita (5.1% fat)
ovaltine (3.8% fat)
biscuits

thirty-five transplanted there who received intravenous feeding were two children aged 7 and 11. Eight patients of the thirty-five were aged 16 years or less. The majority of units in Europe transplanting children use routine intravenous feeding. Since using routine intravenous feeding a weight loss greater than 5% in the French transplant children is unusual (Gluckmann 1979). A major difference between the Royal Marsden and other centres is that cyclosporin-A not methotrexate, is used to prevent GVHD. Little severe mouth ulceration is seen as a result, whereas in Seattle, 80-90% of patients have severe mucositis. This mucositis is particularly common in the patients transplanted for acute leukaemia because of the combined effect of total body irradiation and methotrexate.

Using these oral foods, supplemental foods and intravenous feeding as described, the infants gained weight and older children who did not develop acute GVHD and received intensive feeding support lost about 5% of their body weight during the transplant. Those without this intensive support lost 20% of their body weight.

The intention was to supply a pathogen-free palatable diet for the children undergoing transplantation. There were no facilities to prepare this elsewhere and bring it ready for eating to the child and so a pre-sterilised food supply was established with foods being cooked in the protected environment. A considerable choice of foods became available giving a widely varied diet. The combination of these foods with additional nutritional encouragement prevented an unacceptable weight loss.

## EVALUATION OF THREE PROPHYLACTIC ANTIMICROBIAL REGIMENS

### Introduction

The combination of oral framycetin, colistin and nystatin (FRACON, table 20 ) has been useful in preventing infection in patients undergoing remission induction of AML within a protected environment (Storring et al 1977). A similar regimen (NEOCON, table 20 ) using half the quantity of antibiotics contained in FRACON was described as being of equal efficacy by Watson and Jameson (1979), but an earlier variation of FRACON, AFRACO, was used from 1976 for children undergoing marrow transplantation at Westminster Children's Hospital. At first framycetin, colistin

and nystatin were commenced simultaneously but in the first three children thus treated, candida spp. persisted in their stools and the stools of one child grew only candida spp. on culture. For the 17 subsequent children, the antifungal agent was commenced four days before framycetin and colistin and oral amphotericin-B replaced nystatin as the antifungal agent, thus giving the acronym AFRACO. (Table 20 ). The bone marrow transplant units at Seattle and at the Royal Marsden Hospital, London use respectively gentamicin, vancomycin and nystatin (GVN) and NEOCON. Both regimens include oral amphotericin although the acronyms do not reflect this. Both units also commence the antifungal agents 48 hours before the antibiotics.

A further regimen in which there is increasing interest is co-trimoxazole plus antifungal agents (TSN, table 20 ). Interest in co-trimoxazole as a prophylactic agent to prevent infection in immunosuppressed patients stemmed from Hughes et al (1977) who reported that in addition to effectively protecting children with leukaemia against pneumocystis carinii, the children taking co-trimoxazole had fewer intercurrent infections. Long term use of sulphonamides or co-trimoxazole in patients with urinary infections had not led to problems due to multi-antibiotic resistant organisms and early work using co-trimoxazole to prevent infection during periods of severe neutropenia showed encouraging results. The present results of a randomised prospective trial comparing TSN with NEOCON show that although useful, co-trimoxazole has certain disadvantages in children and adults undergoing bone marrow transplantation because of acute myeloid leukaemia (AML).

AFRACO, FRACON/NEOCON and TSN as used for children undergoing marrow transplantation can be compared to determine whether any regimen has particular benefits within the paediatric population and how the results obtained from patients aged 16 years or less compare with results obtained from adults.

Patients and regimens

The prophylactic antimicrobial regimens are detailed in table 20 . AFRACO was given to all children undergoing bone marrow transplantation at Westminster Children's Hospital (WCH). The patients' diagnoses included severe combined immune deficiency (5 patients), acquired aplastic (7 patients) and Fanconi's anaemia

Table 20

ORAL PROPHYLACTIC ANTIMICROBIAL REGIMENS

| | |
|---|---|
| FRACON | Framycetin 500 mg qds |
| | Colistin $1.6 \times 10^6$ units qds |
| | Nystatin $0.5 \times 10^6$ units (tablets) qds |
| | $0.5 \times 10^6$ units (syrup) qds |
| | Amphotericin B lozenges 10 mg qds |
| AFRACO | Amphotericin B 200 mg qds |
| | Framycetin 500 mg qds |
| | Colistin $1.6 \times 10^6$ units qds |
| NEOCON | Neomycin 500 mg bd |
| | Colistin $1.6 \times 10^6$ units bd |
| | Nystatin $0.5 \times 10^6$ units (tablets) bd |
| | Nystatin $0.1 \times 10^6$ units (syrup) bd |
| | Amphotericin B 200 mg qds |
| | Amphotericin B lozenges 10 mg qds |
| TSN | Trimethoprim 160 mg bd |
| | Sulphamethoxazole 800 mg bd |
| | Nystatin $0.5 \times 10^6$ units (tablets) bd |
| | Nystatin $0.1 \times 10^6$ units (syrup) bd |
| | Amphotericin B 200 mg qds |
| | Amphotericin B lozenges 10 mg qds |

All drugs given orally. Quoted doses are adult equivalents

(3 patients) and acute lymphoblastic leukaemia (2 patients). The patients at the Royal Marsden Hospital (RMH) received FRACON, NEOCON or TSN. All patients receiving TSN suffered from acute myeloid leukaemia (AML) as did 11 of the 17 transplanted children receiving FRACON/NEOCON; of the other six children, two had idiopathic aplastic anaemia and four had acute lymphoblastic leukaemia. Twenty-six of 28 adults received FRACON/NEOCON or TSN in the course of their treatment for AML; the other two being transplanted because of chronic myeloid leukaemia. All patients were cared for in a protected environment and received specially prepared food.

The patients receiving AFRACO form too heterogeneous a group to usefully compare data relating to infection or the incidence of fever as many received prophylactic granulocyte transfusions. It is equally inappropriate to compare their requirement for additional therapeutic antibiotics. However data relating to the effects of oral AFRACO on the stool flora may be compared with the results of giving FRACON, NEOCON or TSN despite differing institutions and diagnoses as stool culture methods were similar. The microbiological and clinical results of giving NEOCON or TSN in a prospective randomised trial are directly comparable. Patients who could not tolerate their prophylactic regimen were excluded, but those who had their regimen withdrawn for clinical reasons are included in the stool microbiological results up to the time of withdrawal.

## Microbiological aspects

### Methods

Stools were obtained three times (WCH) or twice (RMH) each week. They were transported to the laboratory and cultured within four hours. A weighed gram of stool was added to 9 mls sterile water and vigorously shaken for 5-10 seconds. A standard 10 $\mu$l loop of the suspension was streaked onto blood agar plus neomycin, MacConkey agar, and Sabouraud's medium. Following overnight incubation at 37$^{o}$C under both aerobic and anaerobic conditions the plates were read and such further identification of colonies as was appropriate carried out. Previous experience and a small repeat study showed that a one in ten dilution of

stool gave similar qualitative microbiological results to dilutions ranging from one in a hundred to one in one hundred-thousand. There appeared to be no additional organisms cultured at these higher dilutions suggesting that neither antibiotic in the stool nor overgrowth by aerobes was exerting a major influence on the culture results.

Organisms were considered to be suppressed in the stool if they were not grown on culture as described above. Consistent suppression was defined as the organism or group of organisms not being cultured at any time during the study period. Stools were considered bacteriologically acceptable if no enterobacteriaceae, pseudomonads or well recognised pathogens could be cultured from the stool and mycologically satisfactory if no fungi were cultured using the above techniques. Bacteria considered acceptable included bacteroides spp., lactobacilli, strep. faecalis and staph. albus. Entirely acceptable stools contained neither fungi nor undesirable bacteria on culture. Stools described as "no growth" gave no bacterial or fungal isolates after 24 hours culture. An isolate was the culturing of a species of micro-organism from a stool and any one stool culture might contain several such isolates. Unacceptable isolates were any species of enterobacteriaceae, pseudomonad, well recognised pathogen or fungus.

## Results

### Microbial suppression

Fungi were consistently suppressed in the stools of 59 (80%) of 74 transplant patients while they occupied a protected environment and received prophylactic oral antimicrobial agents. (table 21). The number who received TSN was rather small to properly compare but TSN was effective in both children and adults in contradistinction to AFRACO and FRACON/NEOCON which both suppressed fungi consistently from the stools of 65% of a heterogenous group of transplant children, compared with 93% of adults.

The stools were consistently bacteriologically satisfactory in 44 (60%) of 74 transplant patients, who received AFRACO, FRACON/NEOCON or TSN. (Table 22). Sixty-one percent of adults had consistently bacteriologically acceptable stools compared

Table 21

CONSISTENT SUPPRESSION OF STOOL FUNGI IN TRANSPLANT PATIENTS

| Regimen | No. of patients | Patients with consistent suppression of fungi (percent) |
|---|---|---|
| AFRACO | 17 children | 11 (65%) |
| FRACON)<br>NEOCON) | 17 children<br>28 adults | 11 (65%)<br>26 (93%) |
| TSN | 4 children<br>8 adults | 4 (100%)<br>7 (88%) |

Table 22

CONSISTENT SUPPRESSION OF UNDESIRABLE BACTERIA IN TRANSPLANT PATIENTS

| Regimen | No. of patients | Patients with consistent suppression of undesirable bacteria (percent) |
|---|---|---|
| AFRACO | 17 children | 10 (59%) |
| NEOCON)<br>FRACON) | 17 children<br>28 adults | 10 (59%)<br>18 (64%) |
| TSN | 4 children<br>8 adults | 2 (50%)<br>4 (50%) |

to 58% of children. AFRACO and FRACON/NEOCON were equally effective in the children.

It has already been shown that FRACON and NEOCON alter the stool flora (Watson and Jameson 1979) to the same extent and therefore these results of microbial suppression bear out the clinical impression that it is more difficult to achieve entirely acceptable stools in children. Despite the heterogeneity of the AFRACO group, their results are similar to the more homogeneous groups receiving FRACON/NEOCON or TSN.

Children undergoing transplantation for acute leukaemia are less likely to achieve consistent entirely acceptable stools than adults (table 23) undergoing transplantation or remission induction, even when the same oral antimicrobial regimen is used. Seven of the children in table 23 were in leukaemic relapse at transplantation but three of these achieved entirely satisfactory stools so there is no prima facie reason that this relapse group have affected the results.

Microbial isolates from stools

The isolates from the stools of transplanted children are shown in table 24. The number of children receiving TSN is too small for real comparison but there is no indication that they have a greater proportion of undesirable isolates than those taking AFRACO or FRACON/NEOCON. The greatest proportion of the stools yielding no growth in culture were in those patients receiving AFRACO but the proportion of unacceptable isolates from the stools of these children was similar to that from those receiving FRACON/NEOCON. All but two of the pseudomonas isolates from the AFRACO group were contributed by one infant. There were more isolates of candida from those receiving FRACON/NEOCON.

Oral dose of antimicrobial agents

The dose of oral amphotericin B prescribed per kg body weight as part of the AFRACO regimen was considered in relation to whether or not fungi could be cultured on any occasion from the children's stools (Table 25). Of ten children who received more than 12 mg/kg/day of oral amphotericin B, one showed the presence of candida spp. on stool culture. Of seven children who received less than 12 mg/kg/day, candida spp.

Table 23

PATIENTS WITH LEUKAEMIA (RMH)

| Regimen | NEOCON FRACON a) | NEOCON b) | NEOCON c) |
|---|---|---|---|
| Procedure | Transplant | Transplant | Remission induction |
| Patients | Children | Adults | Adults |
| No. of patients | 20 | 28 | 50 |
| No. intolerant of regimen | 3 | 2 | 4 |
| Patients evaluable | 17 | 26 | 46 |
| No. with consistent entirely satisfactory stools (percent) | 7 (41%) | 15 (58%) | 34 (74%) |

a c $p < .05$

a b Not significant

Table 24

ISOLATES FROM STOOLS OF TRANSPLANTED CHILDREN RECEIVING DIFFERENT ORAL ANTIMICROBIAL PROPHYLAXIS REGIMENS

| | AFRACO | FRACON/NEOCON | TSN |
|---|---|---|---|
| No. of patients | 17 | 17 | 4 |
| No. of stools | 183 | 96 | 16 |
| No growth | 43 (24%) | 11 (11%) | 0 (0%) |
| No. of isolates of staph. albus | 29 | 5 | 0 |
| lactobacilli | 9 | 22 | 8 |
| bacteroides | 31 | 20 | 4 |
| clostridia | 3 | 6 | 1 |
| strep. faecalis | 23 | 24 | 8 |
| pseudomonads | 14 | 1 | 0 |
| esch coli | 10 | 20 | 5 |
| enterobacteriaceae | 32 | 27 | 1 |
| candida spp. | 10 | 16 | 0 |
| Total isolates | 171 | 124 | 26 |
| Unacceptable isolates (per cent) | 56 (33%) | 44 (35%) | 5 (19%) |

Table 25

TRANSPLANT CHILDREN IN ISOLATION RECEIVING AFRACO
AMOUNT OF AMPHOTERICIN B AND STOOL CULTURE RESULTS

| Name | Diagnosis | Isolation | Amphotericin mg/kg/day | Fungi cultured from the stool |
|---|---|---|---|---|
| AJ | SCID | VTI | 26 | YES |
| LS (1) | SCID | VTI | 56 | NO |
| LS (2) | SCID | VTI | 30 | NO |
| SI | SCID | VTI | 50 | NO |
| JW* | SCID | BN | 38 | NO |
| MR | FA | VTI | 10.6 | NO |
| JW | AA | VTI | 6.1 | YES |
| MB | AA | VTI | 7.2 | YES |
| LP | AA | VTI | 12.8 | NO |
| KG | FA | VTI | 13.2 | NO |
| AU | AA | VTI | 19.2 | NO |
| AD* | AA | VTI | 10 | YES |
| RR* | FA | VTI | 10 | NO |
| CT* | AA | VTI | 20 | NO |
| LA | AA | BN | 16.8 | NO |
| PC | ALL | BN | 8.8 | YES |
| RS | ALL | BN | 9.6 | YES |

* died 24-48 hours before transplant

VTI - Vickers Trexler Isolator

SCID - Severe combined immune deficiency

AA - Aplastic anaemia

BN - Barrier nursing cubicle

FA - Fanconi's anaemia

ALL - Acute lymphoblastic leukaemia

was cultured from the stools of six. Those children whose stools consistently contained only acceptable bacteria received the same amount of framycetin (85 mg/kg/day) and slightly less colistin (98,000 units/kg/day compared to 114,000 units/kg/day) compared to those whose stools were not consistently acceptable (table 26).

Influence of protected environment

AFRACO was used in two environments, an isolator tent and a normal hospital cubicle with such protective measures as were possible including the staff wearing protective clothing and the children receiving specially prepared food. More children in the isolators had entirely acceptable stools (table 27). The proportion of 'sterile' stools was the same in each environment (table 27 ) but greater than the 12% of stools examined when children were taking FRACON/NEOCON in a protected cubicle with filtered air. Patients in the isolator received mean doses of framycetin and colistin of 92 mg/kg/day and 101,000 units/kg /day respectively compared with 73 mg/kg/day and 114,000 units/ kg/day given to those in cubicles. Both groups received similar amounts of amphotericin B (20.8 mg/kg/day and 18.3 mg/kg/day). With such small numbers of patients and disparate diagnoses it is doubtful whether these environmentally induced differences are real.

Table 27

CHILDREN ON AFRACO

| Environment | Patients | Patients with | | |
|---|---|---|---|---|
| | | Bacteriologically acceptable stools | Mycologically acceptable stools | Entirely acceptable stools |
| Isolator | 13 | 7 | 9 | 6 |
| Cubicle | 4 | 3 | 2 | 1 |

STOOLS CULTURED FROM CHILDREN IN DIFFERENT ENVIRONMENTS

| Environment | No. of Stools | No. with no growth |
|---|---|---|
| Isolator | 163 | 39 (24%) |
| Cubicle | 20 | 4 (25%) |

Table 26

TRANSPLANT CHILDREN IN ISOLATION RECEIVING AFRACO
AMOUNT OF ANTIBACTERIAL DECONTAMINATION
AND STOOL CULTURE RESULTS

| Name | Diagnosis | Framycetin mg/kg/day | Colistin x1000 IU /kg/day | Stools acceptable or not on bacterial culture |
|---|---|---|---|---|
| AJ | SCID | 260 | 132 | YES |
| LS (1) | SCID | 140 | 144 | NO (pseudomonas spp) |
| LS (2) | SCID | 116 | 192 | NO (kliebsiella spp) |
| SI | SCID | 128 | 128 | YES |
| JWh* | SCID | 96 | 196 | NO (salmonella spp) |
| MR | FA | 44 | 84 | YES |
| JW | AA | 32 | 30 | NO (coliforms) |
| MB | AA | 36 | 36 | NO (Esch Coli) |
| LP | AA | 96 | 128 | YES |
| KG | FA | 64 | 100 | YES |
| AU | AA | 64 | 96 | YES |
| AD* | AA | 76 | 100 | NO (Esch Coli) |
| RR* | FA | 52 | 52 | YES |
| CT* | AA | 100 | 100 | NO (Proteus spp, Esch Coli) |
| LA | AA | 56 | 168 | YES |
| PC | ALL | 44 | 44 | YES |
| RS | ALL | 48 | 48 | YES. |

* died 24–48 before transplant

For abbreviations and details of isolation see table 25

Comparison between NEOCON and TSN

This prospective randomised study involved both adults and children undergoing marrow transplantation for AML who were allocated at random to receive NEOCON or TSN (table 20 ) as oral antimicrobial prophylaxis within a protected environment. Five children received NEOCON and four received TSN. Twenty-five adults are included in this comparison between the two regimens.

The NEOCON group had a greater proportion of 'sterile' stools, and a lesser proportion of unacceptable isolates from their stools. (Table 28 ). There was no real difference in the proportion of each group who achieved consistently acceptable stools and no patients had consistently sterile stools. Candida spp. was cultured from the stools of two patients on NEOCON and one taking TSN. There were more isolates of enterobacteriaceae from the stools of those taking TSN, and more isolates of bacteroides spp. and staph. epidermidis from those taking NEOCON. The remainder of the isolates in both groups were predominantly strep. faecalis and lactobacilli. A parallel study of the same two prophylactic antimicrobial regimens in adults and children undergoing remission induction of AML within a protected environment showed less difference between NEOCON and TSN (Table 29 ). The percentage of sterile stools was similar in both groups as was the proportion of unacceptable isolates from the stools. More patients receiving NEOCON achieved consistently bacteriologically acceptable stools but two patients taking NEOCON had fungi present on a total of three occasions in their stools. The proportion of isolates of enterobacteriaceae from the two groups was similar with more esch. coli and pseudomonads isolated from the TSN group. Other gram negative bacilli were cultured from the stools of those receiving NEOCON; kliebsiella spp. (9 isolates), proteus spp. (5 isolates).

Even if both the transplant and remission induction stool microbiology results are combined it is difficult to find any significant microbiological difference between the results of the two regimens, except that there is a greater variety of microorganisms cultured from any one stool of those taking TSN. The proportion of patients (44%) achieving entirely satisfactory stools is the same in both groups.

Table 28

STOOL MICROBIOLOGY IN TRANSPLANT PATIENTS WITH AML RECEIVING NEOCON OR TSN

| Regimen | NEOCON | TSN |
|---|---|---|
| Patient numbers | 19 | 15 |
| Stools cultured | 103 | 59 |
| No. with no growth | 13 (13%) | 4 (7%) |
| Total no. of isolates | 144 | 99 |
| isolates/stool | 1.39 | 1.68 |
| unacceptable isolates | 29 (20%) | 28 (28%) |
| No. of isolates of Enterobacteriaceae | 22 (15%) | 22 (22%) |
| Esch. coli | 13 (9%) | 14 (14%) |
| Bacteroides spp. | 21 (16%) | 11 (11%) |
| Staph. epidermidis | 10 (7%) | 3 (3%) |
| Strep. faecalis | 54 (38%) | 26 (26%) |
| Candida spp. | 4 | 2 |
| Pseudomonas spp. | 2 | 2 |
| Lactobacilli | 23 (16%) | 24 (24%) |
| No. of patients with | | |
| consistently sterile stools | 0 | 0 |
| ≥ 25% of stools sterile | 4 (21%) | 3 (20%) |
| consistently acceptable stools (bacteriologically) | 9 (47%) | 9 (60%) |
| consistently acceptable stools (mycologically) | 17 (89%) | 14 (93%) |
| consistently acceptable stools (entirely) | 8 (42%) | 9 (60%) |

Table 29

STOOL MICROBIOLOGY IN PATIENTS UNDERGOING REMISSION INDUCTION OF AML AND RECEIVING NEOCON OR TSN

| | NEOCON | TSN |
|---|---|---|
| Patient numbers | 19 | 16 |
| Stools cultured | 99 | 99 |
| No. with no growth | 9 (11%) | 13 (13%) |
| Total no. of isolates | 134 | 171 |
| isolates/stool | 1.35 | 1.73 |
| unacceptable isolates | 29 (22%) | 38 (22%) |
| No. of isolates of | | |
| Enterobacteriaceae | 25 (19%) | 31 (18%) |
| Esch. coli | 10 (7%) | 24 (14%) |
| Bacteroides spp. | 19 (14%) | 16 (9%) |
| Staph. epidermidis | 5 (4%) | 6 (4%) |
| Strep. faecalis | 65 (49%) | 63 (37%) |
| Candida spp. | 3 | 0 |
| Pseudomonads | 1 | 6 |
| Lactobacilli | 15 (11%) | 25 (15%) |
| No. of patients with | | |
| consistently sterile stools | 0 | 0 |
| more than 25% of stools sterile | 4 (21%) | 4 (25%) |
| consistently acceptable stools (bacteriologically) | 10 (53%) | 5 (31%) |
| consistently acceptable stools (mycologically) | 17 (89%) | 16 (100%) |
| consistently acceptable stools (entirely) | 9 (47%) | 5 (31%) |

Discussion

Priesler et al (1970) using a rotating antimicrobial regimen cultured fungi from 49% of the stools and Bodey and Rosenbaum (1974) cultured fungi from 81% of the stools when patients were prescribed PPVF and from 56% of the stools when the patients received GVN. Their patients were undergoing remission induction of AML usually in a protected environment and in the similar group of patients reported here, fungi were cultured from 2% of the stools of those receiving NEOCON and never from the stools of those receiving TSN. Transplant recipients had higher rates of fungal isolation, respectively 5% for AFRACO and 17% for FRACON/NEOCON, but these isolations were almost entirely confined to the children. There was no difference between AFRACO and FRACON/NEOCON in the ability of either regimen to give consistent elimination of fungi in children.

. The dose of amphotericin B would seem to be important in that 90% of those receiving 12 mg/kg/day or greater had consistent elimination of fungi from the stools compared with 14% of those patients who received less than 12 mg/kg/day. Adults receiving NEOCON or TSN regimens receive 880 mg per day and should therefore receive adequate if weighing less than 74 kg. If the dose should be based on surface area then a lesser amount per kg will suffice in an adult compared to a child of less than nine years.

Why it should be more difficult to obtain consistently acceptable stools in children with leukaemia undergoing marrow transplantation than similar adults is difficult to explain. The major difference is in the incidence of fungal suppression. If the candida isolates (table 24 ) are disregarded the percentage of unacceptable bacterial isolates is 23% in those children transplanted using FRACON/NEOCON, a figure similar to the overall 18% of unacceptable bacterial isolates found in the NEOCON arm of the prospective trial involving transplant patients (table 28 ) and the 20% found in the parallel study of those undergoing remission induction. These differences are minor and it is the different incidence of fungal isolation which mainly causes the . greater difficulty in obtaining consistent acceptable stools from children.

Consistently sterile stools were not achieved in any of the patients described here but were achieved in 9% of those taking Priesler's rotating regimen and 26% of those taking GVN from Bodey and Rosenbaum (1974). Whether the stools are sterile or not depends on many factors including whether the oral antimicrobial agents have been eluted from the stool prior to culture. It would seem more important that the bacteria present are acceptable in that if they are only low-grade pathogens the chances of infection arising are lessened.

Guiot and Van Furth (1977) introduced the term 'partial antibiotic decontamination' (better named 'selective antibiotic decontamination') to describe their objective of eliminating only pathogens and not achieving sterile stools. This had been the practical aim of all workers who accepted that sterility of the stools was either very difficult to achieve or else inadvisable on account of possible super-infection or patient symptoms. Selective decontamination preserves the resistance to colonisation described by Van der Waaij et al (1971). They reported that germfree mice were difficult to contaminate with oral esch. coli, pseudomonas spp. or kliebsiella spp., providing the germfree mice had previously been contaminated with the stools of conventional but antibiotic decontaminated mice. The more lactobacilli and bacteroides spp. then present in the stools of the mice, the greater was the resistance of these mice to colonisation by enterobacteriaceae. The stool flora of patients given neomycin, polymixin and antifungal agents also protects germfree mice against such colonisation (Heit et al 1980).

Preservation of the stool anaerobes and strep. faecalis is associated with less diarrhoea and fewer isolates of candida spp. or enterobacteriaceae. This has been associated with a reduction in GVHD in some groups of transplant patients. (Vossen 1980). As vancomycin will remove bacteroides spp. as well as pathogens and therefore seems inadvisable, there seems little gain in achieving sterile stools at present, which is the intention with GVN regimens but is neither the intention, nor would seem possible with most other regimens. However suppression of pathogens is a reasonable and achievable aim.

Of the randomised remission induction patients (table 29) receiving NEOCON, 47% had consistent suppression of pathogens

compared to 31% of those receiving TSN. Of transplanted patients, 59% receiving AFRACO, 46% of those receiving FRACON/NEOCON and 50% receiving TSN had consistent suppression of pathogens. In the only reported transplant series Buckner et al (1978) do not report suppression of pathogens but found 20% of their transplanted patients receiving GVN had consistent suppression of all faecal flora. These patients with satisfactory stools were all among the 25% of patients who took and retained all their prescribed GVN. Authors using oral non absorbable antimicrobial prophylaxis in remission induction of AML have reported suppression of pathogens in 0% (BNNP, Dietrich et al 1977) 25-33% (GVN, Bodey and Rosenbaum 1974; Levine et al 1973), and more than 90% of patients (GVN, Schimpff et al 1975; FRACON, Storring et al 1977; NEOCON, Watson and Jameson 1979). The definitions of a pathogen vary slightly from series to series.

The difference in the proportion of patients with consistent stool pathogen suppression by NEOCON (90%, reported by Watson and Jameson 1979 and 47% in this present study) are significant but are related to methodology. In this present study all stools passed more than 36 hours after commencing the antibacterial agents are considered whereas in the previous study a trend was allowed to develop and the steady state reached constituted whether or not the stools were acceptable.

The greatest and rarely discussed variable (compliance) was well shown by Buckner et al (1978) who described how 73% of their patients did not take all their GVN and that 17% of patients took less than 10% of their prescribed antimicrobial agents. It was not possible to determine this within the NEOCON/ TSN study but when using AFRACO and studying six children intensively, a compliance ratio of about 88% was found for amphotericin and framycetin but only 65% for colistin.

No difference was found in the quantities of framycetin and colistin ingested per kg. body weight in those whose stools were or were not bacteriologically acceptable. This is in keeping with the finding that the change from FRACON to NEOCON which halved the effective doses of neomycin and colistin did not result in a great change in the stools. Indeed it is possible that a further reduction in the amount of NEOCON is practicable,

but vomiting one dose of a twice daily regimens means that little prophylaxis will have occurred over a 12 hour period compared to six hours if a dose of a four times a day regimen is vomited.

Patients in isolators receiving AFRACO were more likely to have entirely acceptable stools than patients in cubicles but the numbers are too small to draw a firm conclusion. Since the percentage of sterile stools was the same in each group and two of the cubicle patients received inadequate amounts of amphotericin, there is no evidence that the exigencies of the isolator contributed to the achieving of a more acceptable stool flora.

From assessments of stool microbiology, there is little to choose between AFRACO and FRACON/NEOCON in creating a more acceptable stool microbial population. FRACON and NEOCON give very similar results but the dose of amphotericin needs to be at least 12 mg/kg/day. TSN seems to give fewer microbiologically acceptable stools though only one of 31 patients had candida in their stools compared with 4 of 38 who received NEOCON, and a greater proportion of transplant recipients had consistently acceptable stools while taking TSN. All these regimens compare well (especially in cost) with the standard North American regimen GVN. Children do seem to be more difficult to achieve consistent satisfactory stools, mainly because of fungal isolates and since fungal isolates are so infrequent when taking TSN, the use of co-trimoxazole should achieve useful prophylaxis.

Prevention of infection by prophylactic antimicrobial agents

The object in using prophylactic oral antimicrobial agents is to reduce the risk of infection in the severely neutropenic patient. FRACON has been shown to be effective within a protected environment (Storring et al 1977) and the change to NEOCON, also in patients undergoing remission induction of AML, did not lead to more days with fever or days when therapeutic antibiotics were required. In addition, none of the 34 patients receiving NEOCON died of infection (Watson and Jameson 1979). TSN was studied in a randomised prospective trial to determine whether this absorbable antimicrobial agent was more effective than the non-absorbable NEOCON at preventing infection during

marrow transplantation for AML which is associated with a much shorter period of neutropenia than remission induction of AML.

Broad comparison can be made with previously published results of the prophylactic antimicrobial regimens used in AML remission induction but nothing has been published discussing the role of absorbable prophylactic antimicrobial agents in bone marrow transplantation. It is not possible to discuss the AFRACO regimen in terms of infection prevention since the 17 children were a heterogeneous group without a control series.

Definitions and methods

All patients admitted for remission induction or bone marrow transplantation because of AML who were nursed in protective isolation were allocated at random to receive either NEOCON or TSN (table 20 ). If patients were known to be intolerant of co-trimoxazole before the randomisation procedure (coin-toss), they were not randomised but received NEOCON and were excluded from the study. If it became known after randomisation that they, or in the case of marrow transplant patients their donors, were intolerant of co-trimoxazole, they were withdrawn from the study, received NEOCON and were disregarded. Patients were considered to be on study 24 hours after commencing oral antibacterial agents which were in all cases commenced 48 hours after the antifungal agents. All patients were seen at least three times per week by the same observer.

A fever day was a day when the patient's temperature was at least $38^{o}C$ on two occasions or $38.5^{o}C$ or greater on one occasion. An antibiotic day was one on which therapeutic antibiotics were administered. Antibiotics already commenced before entry to the trial were disregarded. An antibiotic course was a single or a combination of antibiotics given for a discrete period of time and altered in the light of subsequent antibiotic sensitivity reports on any cultured organism. Circumstances requiring a change of antibiotics not based on culture or antibiotic sensitivity evidence were considered to be an additional course of antibiotics. Bacteraemia was diagnosed only when proven by blood culture results and blood cultures were only performed when clinically indicated. The isolation of a well recognised pathogen from one blood culture bottle was

significant but less definite pathogens e.g. staph. epidermidis required that at least two blood culture bottles contained the organism and that a clinical response followed appropriate therapy. Local infections required local inflammation, pain tenderness and redness to be present and were considered proven when an appropriate organism was cultured from the site. Local candidiasis and viral infections were excluded from this definition.

Most patients undergoing remission induction were inpatients for four days before entering the study and had received such blood and platelet support as required by their presenting haematological indices. Marrow transplant patients were well and in remission and required no immediate support. Stool characteristics were as reported by the patients. Those not speaking adequate English were excluded from the stool assessments. Anti-diarrhoeal agents were used liberally when indicated in all patients. Patients came off study when isolation was discontinued which usually coincided with discharge from hospital.

Of 9 patients age 16 or less, 5 received NEOCON and 4 received TSN. These small numbers do not make it appropriate to present the paediatric results separately. Thirty-seven patients undergoing marrow transplantation were randomised, 21 to receive NEOCON and 16 to receive TSN. Two patients vomited their NEOCON and three were similarly withdrawn from the group receiving TSN, two because of a rash and one because of repeated vomiting of all preparations of Septrin. An additional three patients had TSN discontinued, two who developed intercurrent renal failure associated with the mismatched graft syndrome (Powles et al 1980) and one on account of haematological toxicity. Twenty-nine patients were therefore evaluated, 19 who received NEOCON and 10 who received TSN.

Details of the diagnoses, sex distribution, age and time on study are shown in table 30 as are the duration and degree of neutropenia in each group. Those receiving NEOCON spent an average of 14.3 days with less than $0.5 \times 10^9$ neutrophils/l compared with 13.2 days with a similarly low neutrophil count in those taking TSN.

Table 30

RANDOMISED PATIENTS UNDERGOING MARROW GRAFTING

| | NEOCON | TSN |
|---|---|---|
| Patients randomised | 21 | 16 |
| Patients withdrawn | 2 | 6 |
| Patients evaluable | 19 | 10 |
| Diagnoses AML | 17 | 10 |
| CGL | 2 | 0 |
| Males | 11 | 8 |
| Females | 8 | 2 |
| Mean age | 25.4 yrs | 23.2 yrs |
| range | 11–46 | 10–45 |
| Mean days on study/patient | 32.1 | 18.8 |
| range | 18–53 | 25–35 |
| Total days on study | 609 | 288 |
| Duration of neutropenia | | |
| Days with neutrophils | | |
| $< 0.1 \times 10^9/l$ | 129 (21%) | 65 (23%) |
| $0.1–0.5 \times 10^9/l$ | 142 (23%) | 67 (23%) |
| $0.501–1.0 \times 10^9/l$ | 115 (19%) | 42 (15%) |
| $> 1.0 \times 10^9/l$ | 223 (37%) | 114 (40%) |

## Results

### Fever days and days on therapeutic antibiotics

The percentage of fever days was similar in both groups (table 31 ) but 15 of the febrile days in those receiving TSN were contributed by one patient who had indolent GVHD. The group receiving TSN spent two days in every ten receiving additional therapeutic antibiotics compared with the NEOCON group who received additional antibiotics on over four days in ten. The TSN patients had half as many courses of antibiotics per 100 days as the NEOCON group and many more patients receiving TSN required no therapeutic antibiotics.

### Infections

Bacteraemia occurred in five of 19 patients receiving NEOCON and one of 10 patients taking TSN. Table 32 also shows the organisms involved. The incidence of bacteraemia while receiving TSN was less than half that found in patients receiving NEOCON. Sixteen proven local infections and eleven clinical local infections were found, three-quarters in the group taking NEOCON (table 33 ). Two patients had more than one proven local infection. One local infection was associated with a bacteraemia. The distribution of sites of local infection, proven or not, were similar in both groups with the exception of the urinary tract where there were four times as many proven infections per patient in those receiving NEOCON. No patient receiving TSN had an infection due to staph. aureus and amongst all patients only one infection was proven to be due to gram negative bacilli. One NEOCON patient received two leukocyte transfusions on account of pneumonia due to streptococcus faecalis.

The data showing how many of each group had less than three days with fever, no microbiologically proven infection and no additional therapeutic antibiotics given are shown in Table 33a. Each circumstance shows a benefit for those patients receiving TSN, but in each case p is greater than 0.05. One patient taking NEOCON fell into all the above categories compared with three who received TSN.

Table 31

FEVER DAYS AND ANTIBIOTIC THERAPY IN TRANSPLANT PATIENTS

| | NEOCON n=19 | TSN n=10 |
|---|---|---|
| Total days | 609 | 288 |
| Days with fever | 58 9.5% | 28 9.7% |
| Days on therapeutic antibiotics | 256 (42%) | 58 (20%) |
| Courses of antibiotics/100 days | 5.1 | 2.8 |
| Patients receiving no additional antibiotics | 1 (5%) | 5 (50%) |
| Patients on antibiotics at entry | 2 | 1 |

Table 32

BACTERAEMIA IN TRANSPLANT PATIENTS

| | NEOCON | TSN |
|---|---|---|
| No. of bacteraemia | 5 | 1 |
| Bacteraemia/100 days | 0.82 | 0.35 |
| Bacteraemia/patient | 0.26 | 0.1 |
| Causative organism | | |
| Staph. epidermidis | 2 | 1 |
| Staph. aureus | 1 | 0 |
| Pseudomonas aeruginosa | 1 | 0 |
| diphtheroids | 1 | 0 |

Table 33a

LOCAL INFECTIONS IN TRANSPLANT PATIENTS

| Regimen | NEOCON n=19 | n=10 TSN |
|---|---|---|
| No. of local infections | 21 | 6 |
| No. of proven local infections | 14 | 2 |
| Proven local infections/100 days | 2.29 | 0.7 |
| No. of patients with local infection | 14 | 4 |
| Patients receiving granulocyte transfusions | 1 | 0 |

Table 33b

SITE AND CAUSE OF LOCAL INFECTIONS IN TRANSPLANT PATIENTS

| Regimen | NEOCON | | TSN | |
|---|---|---|---|---|
| | | proven | | proven |
| urinary tract | 8 | 8 | 1 | 1 |
| upper respiratory tract | 2 | 1 | 2 | 0 |
| dental | 1 | 1 | 0 | 0 |
| skin and soft tissue | 8 | 3 | 2 | 1 |
| lower respiratory tract | 2 | 1 | 1 | 0 |
| (perianal) | 2 | 0 | 0 | 0 |
| strep. faecalis | | 8 | | 1 |
| staph. epidermidis | | 1 | | 1 |
| staph. aureus | | 3 | | 0 |
| pseudomonas spp. | | 1 | | 0 |
| diphtheroid spp. | | 1 | | 0 |

PATIENTS WITH FEW INFECTIVE PROBLEMS Table 33c

| Regimen | NEOCON | TSN |
|---|---|---|
| No. of patients | 19 | 10 |
| Patients with ⩽2 days of fever | 10 (53%) | 7 (70%) |
| Patients with no proven infection | 5 (26%) | 7 (70%) |
| Patients receiving no therapeutic antibiotics | 1 (5%) | 5 (50%) |

Haematological reconstitution and support

The number of transplanted nucleated stem cells/kg. recipient body weight was similar in both groups and although those taking TSN required 36 hours longer on average to attain $0.5 \times 10^9$ polymorphs/l, this difference is not significant (table 34 ). Patients taking TSN received 10 units of blood and 56 units of platelets per 100 days on study compared to 11.4 units of blood and 77 units of platelets per 100 days required by those taking NEOCON (table 35 ).

Subjective stool changes

Patients receiving TSN reported that their stools were loose, very loose or diarrhoeal on 27% of days and that they passed no stool on 41% of days (table 36 ). They passed on average six stools in every week. Those taking NEOCON reported loose, very loose or diarrhoeal stools on 41% of days and absolute constipation on 33% of days. On average they passed nine stools in each week. One of nine receiving TSN spent more than half their days with loose, very loose or diarrhoeal stools compared with four of sixteen receiving NEOCON.

Reliable data on weight loss were available for 22 patients. The results were similar with either antimicrobial regimen (table 37 ). Overall, 18% of patients showed a net weight gain between admission and discharge and despite no intravenous feeding being used on any of these patients the mean weight loss of those who lost weight was just over 8% of their admission weight. Those 16 years of age and under lost on average 11.2%

Table 34

HAEMATOLOGICAL RECONSTITUTION POST BONE MARROW TRANSPLANTATION

| Regimen | NEOCON | TSN |
|---|---|---|
| Nucleated stem cells transferred x $10^8$/kg. recipient body weight | 3.24 | 2.92 |
| Mean days post transplant to 0.5 x $10^9$ polymorphs/l (range) | 14.5 (12-26) | 16.1 (12-26) |

Table 35

HAEMATOLOGICAL SUPPORT REQUIRED DURING BONE MARROW TRANSPLANTATION

| Regimen | NEOCON | TSN |
|---|---|---|
| No. of patients | 19 | 10 |
| Units of blood/patient | 3.6 | 2.95 |
| Units of blood/100 days | 11.35 | 10.0 |
| Units of platelets/patient | 24.7 | 16.0 |
| Units of platelets/100 days | 77.2 | 55.5 |

Table 36

STOOLS IN TRANSPLANT PATIENTS

| Regimen | NEOCON | TSN |
|---|---|---|
| No. of patients evaluable | 16 | 9 |
| No. of days observed | 355 | 200 |
| No. of stools passed | 455 | 171 |
| Stools passed in each 7 days | 9 | 6 |
| Days with absolute constipation | 116 | 81 |
| stools firm | 18 | 15 |
| normal | 29 | 29 |
| soft | 30 | 8 |
| loose | 50 | 20 |
| very loose | 30 | 9 |
| diarrhoea | 65 | 24 |
| % of days with loose, very loose or diarrhoeal stool | 41% | 27% |
| % of days with absolute constipation | 33% | 41% |

Table 37

WEIGHT CHANGES DURING TRANSPLANTATION

| Regimen | NEOCON | TSN |
|---|---|---|
| No. of patients evaluable | 13 | 9 |
| No. gained weight | 3 | 1 |
| No. maintained weight | 0 | 0 |
| No. lost weight | 10 | 8 |
| Mean weight loss | 5.48 kg | 4.16 kg |
| Mean weight loss as % of admission weight | 8.8% | 7.3% |

and the adults who lost weight lost 7% of their admission weight.

Discussion

Two patients were withdrawn from the 21 who were randomised to receive NEOCON. Both suffered repeated vomiting which, although possibly aggravated by TBI and the use of cyclosporin-A resolved when NEOCON was discontinued. This proportion (9.5%) is similar to the 11% of 46 patients who were intolerant of FRACON (Storring et al 1977) and the 11% of 38 patients who were intolerant of NEOCON (Watson and Jameson 1979). In the only reported series of patients undergoing transplantation (Buckner et al 1978), 17% of 45 patients took less than 10% of their GVN antimicrobial regimen and clearly found GVN unacceptable. Six patients were withdrawn from the 16 who received prophylactic TSN. Two developed a rash, an incidence of 12.5% which compares with the 12% incidence of rashes reported by Gurwith (1978) using co-trimoxazole for his remission induction patients and more generally quoted data. One had repeated vomiting of co-trimoxazole tablets, dispersable tablets and elixir. One patient had co-trimoxazole discontinued following failure of a first transplant to engraft. At that time free circulating antibodies to trimethoprim were detected in the patient's serum (Claas 1980) which were not present in serum stored before commencing co-trimoxazole. These antibodies were undetectable two months after discontinuing co-trimoxazole. No other tested patients had these antibodies. A second graft from the same donor was successful and subsequent culture of the patient's bone marrow showed this to be suppressed by the addition of both components of co-trimoxazole to the medium. Two further patients randomised to receive TSN received a transplant from a one-haplotype identical donor and developed renal failure as part of the mismatched graft syndrome (Powles et al 1981). Co-trimoxazole was discontinued on account of the renal failure. These six patients withdrawn from those randomised to receive TSN constitute 37.5% of the randomised patients. Unacceptable effects of co-trimoxazole were directly responsible for four (25%) patients being withdrawn.

A rash is one of the cardinal signs of GVHD and thus confusion could arise as to whether GVHD or co-trimoxazole was

responsible for a rash. Biopsy appearances of GVHD should leave little doubt if GVHD is the cause of a rash but negative results will not exclude the development of GVHD.

The evaluable patients were directly comparable in distribution of diagnosis, sex, age and time spent on study. The mean time spent by all the patients with a polymorph count of less than $0.1 \times 10^9/l$ was 6.7 days. This, and the mean days with a polymorph count of less than $0.5 \times 10^9/l$ (13.9 days) is very much shorter in patients transplanted because of leukaemia than those with severe aplastic anaemia where not only is there no early period following immunosuppression during which there are adequate numbers of circulating granulocytes but this period of severe neutropenia will have already persisted for some weeks. Since the incidence of infection rises as the duration of neutropenia extends (Hersh et al 1965; Bodey et al 1966), reported transplant series combining patients with aplasia and patients with leukaemia are not directly comparable with the results of this trial. In addition, rejection of the transplant was until recently common in aplastic patients, further prolonging neutropenia in the aplastic patients. The pre-graft state of the marrow also slightly affects the incidence of infectious complications. Patients transplanted in leukaemic relapse at Seattle had more infections per patient than those transplanted in remission whether or not a protected environment with prophylactic antimicrobial agents was employed. (Buckner et al 1978).

If the patient who had 15 days of fever due to GVHD is excluded, those patients taking TSN had half the number of fever days experienced by those taking NEOCON. The median number of fever days, one, was the same with each regimen. Fever may be caused by circumstances other than infection, e.g. GVHD, reaction to blood product transfusions and endotoxins from bowel organisms. The incidence of histologically proven GVHD was about 25% in both groups and febrile reactions to blood products were not particularly common partly because intravenous hydrocortisone and chlorpheniramine were given before each transfusion. Access by endotoxin may be facilitated by the changes in the gut secondary to TBI and hepatic neutral-

isation of endotoxin impaired by radiation or cyclosporin-A induced hepatitis but these effects would have been common to both groups. Fever was not due to leukaemia as all patients were in remission when transplanted.

At Seattle (Buckner et al 1978) the protected patients each spent over twice as long with a neutrophil count of less than $0.1 \times 10^9/l$ as in this study and 28% of their days (mean 12.6 days per patient) were days on which they were febrile ( $\geqslant 38.3^{\circ}C$). The patients discussed here spent 9.5% of their days febrile, a mean of 2.9 days per patient. This paucity of fever is rather surprising, given that all patients had a general anaesthetic for insertion of a central venous catheter with a subcutaneous tunnel; each received about 19 separate transfusions of blood products; underwent total body irradiation and spent an average of 13.9 days with a neutrophil count of less than $0.5 \times 10^9/l$. In addition 8 (27%) suffered some degree of histologically proven GVHD whilst under study, but of the 45 patients described by Buckner 58% suffered GVHD which was implicated in the deaths of 15% of those affected.

Patients undergoing remission induction of AML and taking FRACON in a protected environment spent a mean of 28.4 days with a neutrophil count of less than $0.5 \times 10^9/l$ and each had 7 days of fever (Storring et al 1977) in comparison to patients taking NEOCON who had a similar neutropenia for 23 days and were febrile for a mean of 5 days. (Watson and Jameson 1979). This and the subsequent therapeutic antibiotic requirements (table 38) emphasise the relative simplicity of this aspect of transplantation to the remission induction of AML.

Therapeutic antibiotics administered will depend somewhat on the policies of the individual unit. Gentamicin and carbenicillin were commenced if the patient had a fever in excess of $38^{\circ}C$ for four hour, or had a clinically diagnosed infection which might or might not become microbiologically confirmed. Cefuroxime was given in place of or in addition to carbenicillin if surveillance cultures with antibiotic sensitivities had suggested this would be useful and cloxacillin was given in addition to gentamicin and carbenicillin to treat cutaneous infections. Antibiotics were administered for about

Table 38

FRACON/NEOCON FOR PATIENTS WITH AML

| Procedure | RI FRACON (Ref 1) | RI NEOCON (Ref 2) | RI NEOCON (Ref 3) | BMT NEOCON (Ref 4) |
|---|---|---|---|---|
| No. of patients | 46 | 34 | 18 | 19 |
| Mean days on study | 39 | 33.5 | 41 | 32.5 |
| Mean days with $\ll 0.5 \times 10^9$ neutrophils/l | 28 (72%) | 23 (68%) | 27 (68%) | 143 (45%) |
| febrile days/ patient | 7 (18%) | 4.7 (14%) | 6.6 (16%) | 3.1 (9.5%) |
| days on therapeutic antibiotics | 18 (45%) | 16 (47%) | 18 (44%) | 14 (42%) |

ref 1 Storring et al (1977)

ref 2 Watson and Jameson (1979)

ref 3 Watson et al (1981)

ref 4 This report

RI - remission induction

BMT - bone marrow transplantation

seven days which frequently coincided with the reappearance of neutrophils in the peripheral blood at day 15 after transplantation since the most usual time for a fever to occur was between days 5 and 8 after grafting. Three patients (two NEOCON; one TSN) were on antibiotics at entry to the trial. One of each group commenced antibiotics because of a fever in relation to the surgical placement of the Hickman intravenous catheter and one was receiving penicillin because of recent mild dental sepsis. These antibiotics were disregarded in the calculation of the results.

Patients receiving NEOCON (tables 31 and 38) each spent 14.3 days (42% of study days) on additional therapeutic antibiotics compared a mean of 5.7 days (20%) for each of the TSN patients, who also received half as many courses of antibiotics. The mean duration of a course of antibiotics was similar in both groups (8.2 and 7.2 days) showing there was no real bias resulting from one group receiving shorter courses of antibiotics than the other. In addition 50% of TSN patients received no additional therapeutic antibiotics compared to 5% of the NEOCON patients.

The comparative percentages of days on antibiotics during remission induction of AML is shown in table 38 . Those undergoing transplantation receive antibiotics on fewer days but the difference is less marked because in all these patients less than one day of fever is sufficient to warrant about 7 days of antibiotic therapy. There are no comparative controlled data including a protected environment in both arms where the antibiotic days are discussed. The data of Buckner et al (1978) who reported antibiotics being administered on 52% of days to their group transplanted in a protected environment is not directly comparable mainly because of the considerably longer period of severe neutropenia in their patients as a result of including both aplastic and relapsed leukaemia patients. They continued antibiotics until the granulocytes reached $0.5 \times 10^9/l$ which added 2 days to the duration of each patient's antibiotic days.

Five bacteraemias occurred in the NEOCON group and one in the TSN group. (Table 32). The incidence per patient or per

100 days on trial was almost three times higher in those receiving NEOCON compared to those receiving TSN and overall six of 29 patients (21%) had a bacteraemia. Buckner et al (1978) reported 10 patients (22%) developing bacteraemia out of 45 undergoing transplantation and in this whole group eight (27%) of 29 were patients with leukaemia. 20% of the small group with leukaemia in remission at the time of their transplant developed a bacteraemia compared with 21% in this Royal Marsden series. However, Buckner et al (1978) had somewhat more stringent criteria for bacteraemia to be diagnosed and amongst the twenty-two microbiologically proven infections in their LAF group only one infection was attributed to staph. epidermidis, whereas three of the six bacteraemias in this trial were due to staph. epidermidis. One bacteraemia was due to gram-negative bacilli, pseudomonas aeruginosa, which could not be cultured from the stools of that patient while receiving NEOCON although he suffered pseudomonas infections during remission induction four months previously and intercurrent stool culture had grown pseudomonas aeruginosa while he was an out-patient.

There were 27 local infections with the incidence of local infection being twice as high in those receiving NEOCON. (table 33a ). However the differences in the site of the local infection between the two groups are not significant although four times as many urinary tract infections occurred in those taking NEOCON. Since the components of the NEOCON regimen are not absorbed unlike the co-trimoxazole of the TSN regimen, it is not surprising that TSN will exert a protective effect on the urinary tract. Two perianal infections occurred, both in those patients taking NEOCON. Storring et al (1977) reported no anorectal infections in 45 remission induction patients taking FRACON and Buckner et al (1978) found only one anorectal infection in 46 transplant patients taking CVN. One concern had been that TSN would not give sufficient reduction in bowel organisms to prevent anorectal infections but TSN gave consistently acceptable stools in 9 (60%) of 15 transplant patients (more than with NEOCON) and gave in addition systemic protection which may explain the lower incidence of anorectal complications and also skin and soft tissue infections.

One patient (taking NEOCON) of the 29 received granulocyte transfusions on account of pneumonia due to streptococcus faecalis. Five of the 46 transplant patients reported by Buckner et al (1978) required granulocyte transfusions compared with 50% of those in a minimally protected environment but not taking prophylactic antimicrobial agents.

No bacteraemias or proven infections were due to enterobacteriaceae and one (3%) due to other gram negative bacilli despite the stools of 10 of 19 taking NEOCON and 6 of 15 taking TSN persistently containing these organisms. The control transplant group reported by Buckner et al (1978) received no oral antimicrobial agents (but had a minimally protected environment) and had 125 proven infections of which 52 (42%) were due to gram negative bacilli. The patients receiving GVN within a protected environment had 22 proven infections of which 2 (9%) were due to gram negative bacilli. GVN, NEOCON and TSN exert considerable protective effects on the incidence of gram negative infection, but overall a greater proportion of patients taking TSN had two or less days of fever, no proven infection and received no therapeutic antibiotics than patients receiving NEOCON (page 118 ).

There was also concern regarding the haematological effects of co-trimoxazole. One patient taking TSN had failure of graft take associated with antibodies to co-trimoxazole and was successfully regrafted from the same donor two weeks after stopping TSN. One patient receiving NEOCON similarly failed to engraft at the first attempt, a circumstance attributed to the use of methotrexate to prevent the mis-matched graft syndrome. Although patients receiving NEOCON received slightly more nucleated stem cells/kg. recipient body weight, the size of the graft was similar in both groups and there was a delay of about 36 hours in the mean time taken to reconstitute to 0.5 x $10^9$ polymorphs/l in those receiving TSN (table 34 ). Folic acid supplements were not given. Platelet counts were more variable and affected by exogenous transfusion and the use of cyclosporin-A but the haematological support required for the two groups showed that less support was required for those receiving TSN. (Table 35). The lower incidence of infection

is the probable explanation for this difference.

One patient taking NEOCON died of the mismatched graft syndrome. No patients died of infection and the death rate of 3.5% on study compares well with the 9% death rate by day 50 (excluding resistant leukaemia) reported by Buckner et al (1978). Of the patients transplanted from a matched sibling entering this study the 50 day survival was 95%.

Patients taking TSN reported less bowel upset than those receiving NEOCON. They passed fewer stools and a greater proportion of stools were either normal, firm or soft in texture. Although patients were given anti-diarrhoeal agents ad libitum there was considerably more fluidity of the stools in those receiving NEOCON. It is surprising that on 35-40% of days there was absolute constipation and bulk laxatives were also freely used, to avoid the dangers of a hard constipated stool causing mucosal abrasion in passage. No specific assessment of patient compliance with the NEOCON or TSN regimens was performed but 75% of the quarter who were fully compliant with the GVN regimen of Buckner et al (1978) achieved their aim of "complete suppression of all faecal flora". In other words 37 of 46 patients (80%) did not achieve this. The aim as far as the stool microbiology was concerned in this study was achieved in 17 (50%) of 34 patients.

The weight loss (mean 8%) is remarkably little and although many factors will interfere with food absorption after a transplant the major difference between this series of patients and others is that cyclosporin-A was used to prevent GVHD, not methotrexate, and consequently all the major oral problems associated with the combination of methotrexate and TBI were avoided.

Compared to NEOCON, patients taking TSN have fewer febrile days, less need for additional therapeutic antibiotics, fewer bacteraemias and fewer local infections both microbiologically proven and clinically observed. Despite their reconstitution after grafting being slightly slower, they required less support with exogenous blood products. They had fewer and more normal stools which were microbiologically similar to those obtained from patients taking NEOCON. They lost less weight and were discharged sooner after their transplant. It would seem that,

if tolerated by the patients and acceptable to the medical staff when taking a whole view of the patient's condition, that TSN is a useful regimen in patients undergoing marrow transplantation.. However if 12% of patients develop rashes and 6% of patients have entirely unacceptable haematological toxicity, TSN will not be an ab initio useful regimen but a useful replacement for other regimens which are not tolerated by individual patients.

SURFACE AND ORIFICE DECONTAMINATION

Although the major endogenous source of infection in neutropenic patients is usually considered to be the gastrointestinal tract, other sites may harbour pathogens which gain access when oral ulceration or mucosal damage is present. Oral antimicrobial agents are primarily given to reduce the alimentary flora which they do to a greater or lesser extent. The non-absorbable preparations have a limited effect on the micro-organisms in other sites but the effect of absorbable preparations e.g. co-trimoxazole has not yet been reported.

Bodey and Rosenbaum (1974) reported very extensive microbiology results from 91 adults with leukaemia and indicated the non-alimentary sites most likely to harbour pathogens and therefore most worthy of further decontaminating measures. (table 39 ). A number of regimens have been used to decontaminate these sites. The most extensive was that of Bodey and Rosenbaum (1974) and the least extensive recently quoted, that of Watson and Jameson (1979) (table 40 ). Both regimens applied to leukaemia patients undergoing remission induction but the regimen of Watson and Jameson (1979) was also used for marrow transplant recipients. A relatively complex regimen was used at Westminster Children's Hospital and its value is worth comparing to the simple regimen used at the Royal Marsden Hospital.

Patients and regimens

All children undergoing marrow grafting in isolator tents at Westminster Children's Hospital were subjected to a rigorous programme of surface and orifice decontamination. Those transplanted at the Royal Marsden Hospital on account of AML received no specific decontamination of their surfaces and orifices but

Table 39

POTENTIAL PATHOGENS IN DIFFERENT SITES

| | Total Isolates | Isolates of potential pathogens | % |
|---|---|---|---|
| Skin | 564 | 265 | 47 |
| Throat | 360 | 162 | 45 |
| Ear | 210 | 30 | 14 |
| Nose | 202 | 45 | 22 |
| Vagina | 95 | 62 | 65 (39% if Esch. coli excluded) |

Bodey and Rosenbaum 1974

Table 40

COMPARISON OF EXTENSIVE AND SIMPLE SURFACE DECONTAMINATION

| | Bodey and Rosenbaum 1974 | | Watson and Jameson (1979) |
|---|---|---|---|
| Action | Contents | Site | |
| Spray q.d.s. | neomycin 100 mg/ml<br>vancomycin 10 mg/ml<br>polymyxin B 5 mg/ml | nose<br>throat | suck amphotericin lozenges 10 mg q.d.s.<br>nystatin elixir 100,000 IU b.d.<br>plenty of mouthwashes with Milton or chlorhexidine 0.02% |
| Apply oihtment q.d.s. | neomycin 50 mg/gm<br>nystatin 25000 units/gm<br>vancomycin 5 mg/gm<br>polymyxin B 2.5 mg/gm | gums<br>ears<br>nose<br>groins<br>perineum | |
| Insert gel b.d. | neomycin 100 mg/gm<br>nystatin 50,000 units/gm<br>vancomycin 10 mg/gm<br>polymyxin B 5 mg/gm | rectum<br>vagina | |
| Use b.d. | iodinated soap or<br>Phisohex or<br>p-300 | Bath | shower daily with Hibiscrub or Baby soap |

a daily shower or bed-bath using 'Baby Soap'. The Royal Marsden regimen is shown in table 40 in which the oral anti-fungal agents formed part of the prophylactic antimicrobial regimens discussed previously. The regimen used at Westminster Children's Hospital is shown in table 41 .

The Hibiscrub bath consisted of Hibiscrub applied to the skin, allowed to almost dry and then washed off. Hibiscrub was not used after total body irradiation in either centre, the effects of the detergent being too drying. For these patients, Johnson's baby soap was used. Chlorhexidine douches to the vagina and nightly instillation of chlorhexidine obstetric cream proved irritating. Half-strength Savlon twice daily was acceptable. All these procedures could be performed by the patient, but nursing supervision gave more efficient care and better microbiological results.

Microbiological surveillance

At Westminster the sites routinely sampled were scalp, hairline, ears, nose and throat, mouth, axillae, umbilicus, groins, foreskin, urine, vagina and rectum if a stool was not available. For the first three weeks swabs from each site were cultured three times each week and subsequently once per week, with half the sites being sampled on Monday and half on Thursday. Swabs to be taken from dry areas were first moistened with peptone water. All swabs were taken before the morning bath about 3 hours after the 6 a.m. spray of the orifices, and cultured for 24 hours on blood agar, MacConkey agar and Sabourauds medium within 4 hours of sampling. At the Royal Marsden Hospital, swabs similarly moistened were taken once each week from the nose, throat, axillae, toes and vagina. Culture conditions were as described above.

Results

Compliance with the procedures

Eight children in isolators at Westminster were studied during a total of 794 days. The data were derived from nursing administration records and may be underestimates. Table 42 shows how often each procedure was recorded as having been carried out. The mean compliance was 76%. The low figure of

Table 41

DECONTAMINATION OF SURFACES AND ORIFICES
AT WESTMINSTER CHILDREN'S HOSPITAL

| | |
|---|---|
| Hibiscrub | (4% chlorhexidine in detergent) bath daily |
| Savlon | (1.5% chlorhexidine in 15% cetrimide) hair washes alternate days. |
| Naseptin | (0.1% chlorhexidine with 0.5% neomycin) to the nares q.d.s. |
| Chlorhexidine | (0.02% aqueous) mouth washes q.d.s. |
| Chlorhexidine | (0.02% aqueous) spray to external auditory canal, throat and foreskin q.d.s. |
| Hibitane | (1% chlorhexidine) dental gel applied to gums b.d. |
| Half-strength Savlon | twice daily to vagina |

Table 42

SURFACE AND ORIFICE COMPLIANCE DATA
EIGHT PATIENTS

| Treatment | Times ordered | Times recorded given | Compliance |
|---|---|---|---|
| Hibiscrub bath | 441 | 322 | 73% |
| Savlon hair wash | 166 | 130 | 78% |
| Naseptin | 1987 | 1578 | 79% |
| Chlorhexidine mouthwash | 2805 | 2217 | 79% |
| Chlorhexidine spray | 1258 | 960 | 76% |
| Hibitane dental gel | 685 | 400 | 58% |
| Overall compliance | 7342 | 5607 | 76% |

58% for the dental gel reflected the children's distaste for this. All these procedures took staff time, were a nuisance and sometimes unpleasant for the children, therefore must be justified microbiologically.

Microbiological results

Table 43 shows the number of sterile cultures from transplanted children in isolator tents and in barrier nursing cubicles. Microorganisms were cultured from 26% of swabs taken from the patients' surfaces in both environments. 75% of the organisms isolated were staph. epidermidis or diphtheroid spp. The orifices were less well decontaminated (table 44 ) although patients in the isolators had a greater percentage of sterile cultures, 46% compared to 9% in the cubicles ($p < .01$).

Table 45 shows the number of sterile cultures in the isolators from infants with severe combined immune deficiency (SCID) and from children with aplastic anaemia or Fanconi's anaemia (AA). The percentage of sterile surface cultures was the same in both groups but more orifice cultures were sterile in the infants ($p > 0.1$). The proportion of sterile stools was the same. The volume of Hibiscrub bath used was similar per unit area of skin. The relative sterility of orifices was greater, so perhaps the greater volume of chlorhexidine spray per unit area contributed to the better score of the infants.

There was a sex difference in the results of the decontamination (table 46 ). Combining the results for SCID and aplastic anaemia showed that males achieved better decontamination ($p < .01$). Table 43 showed that the surfaces of each group had a similar percentage of sterile cultures and that more orifice specimens from the infants were sterile. Since the infant group consisted of 4 females and 2 males this would have skewed the results to favour the female group. In fact, considerably more sterile specimens were found in males than in females (table 46 ). Why the scalp, hair, axillae, nose and throats of females are more difficult to decontaminate than those sites in the male is difficult to explain, but since only 31% of vaginal swabs were sterile this site could well have been acting as a reservoir.

The results from the Royal Marsden Hospital with surveill-

Table 43

SURFACE DECONTAMINATION

| site | n = 16 Isolator patients | | | n = 4 Barrier nursing | | |
|---|---|---|---|---|---|---|
| | No. of specimens | No. sterile | % | No. of specimens | No. sterile | % |
| scalp, hair, forehead | 217 | 182 | 84% | 31 | 25 | 81% |
| ears | 308 | 219 | 71% | 29 | 14 | 48% |
| axillae | 308 | 242 | 79% | 29 | 26 | 90% |
| groins | 302 | 196 | 65% | 26 | 21 | 81% |
| umbilicus | 73 | 57 | 78% | | | |
| eyes | 47 | 33 | 70% | | | |
| total | 1255 | 929 | 74% | 115 | 86 | 75% |

Table 44

ORIFICE DECONTAMINATION

| site | n = 16 Isolator patients | | | n = 4 Barrier nursing | | |
|---|---|---|---|---|---|---|
| | No. of specimens | No. sterile | % | No. of specimens | No. sterile | % |
| nose | 203 | 113 | 56% | 30 | 5 | 16% |
| throat | 199 | 79 | 40% | 30 | 3 | 10% |
| mouth | 108 | 37 | 34% | 31 | 2 | 6% |
| vagina | 83 | 33 | 40% | 16 | 0 | 0% |
| foreskin | 40 | 27 | 68% | | | |
| total | 633 | 289 | 46% | 107 | 10 | 9% |

Table 45a

SURFACE DECONTAMINATION IN ISOLATORS

| | SCID n = 6 | | | AA n = 9 | | |
|---|---|---|---|---|---|---|
| site | No. of specimens | No. sterile | % | No. of specimens | No. sterile | % |
| scalp, hair, forehead | 54 | 44 | 81% | 158 | 133 | 84% |
| ears | 90 | 61 | 68% | 207 | 143 | 69% |
| axillae | 94 | 80 | 85% | 199 | 156 | 78% |
| groins | 86 | 63 | 73% | 202 | 123 | 61% |
| umbilicus | 33 | 27 | 82% | 39 | 28 | 72% |
| total | 357 | 275 | 77% | 805 | 583 | 72% |

Table 45b

ORIFICE DECONTAMINATION IN ISOLATORS

| | SCID n = 6 | | | AA n = 9 | | |
|---|---|---|---|---|---|---|
| site | No. of specimens | No. sterile | % | No. of specimens | No. sterile | % |
| nose | 60 | 39 | 65% | 144 | 53 | 37% |
| throat | 57 | 29 | 51% | 119 | 34 | 29% |
| mouth | 35 | 20 | 57% | 80 | 16 | 20% |
| vagina | 38 | 19 | 50% | 45 | 7 | 16% |
| foreskin | 15 | 12 | 80% | 24 | 14 | 58% |
| total | 205 | 119 | 58% | 412 | 124 | 30% |
| stools | 60 | 18 | 30% | 94 | 24 | 26% |

Table 46

SEX AND THE NUMBER OF STERILE CULTURES IN THE ISOLATORS

| site | Males n = 7 | | | Females n = 8 | | |
|---|---|---|---|---|---|---|
| | No. of specimens | No. sterile | % | No. of specimens | No. sterile | % |
| scalp, hair, forehead | 115 | 109 | 95% | 97 | 68 | 70% |
| axillae | 138 | 128 | 93% | 155 | 108 | 70% |
| groins | 135 | 98 | 73% | 153 | 88 | 58% |
| nose | 84 | 55 | 65% | 91 | 37 | 41% |
| throat | 80 | 35 | 44% | 96 | 29 | 30% |
| stools | 61 | 15 | 25% | 93 | 27 | 29% |
| overall | 613 | 440 | 72% | 685 | 357 | 52% |

ance of five sites in nine children with AML in remission undergoing transplantation are not directly comparable with the results for Westminster Children's Hospital. However, 67% of isolates were staph. epidermidis or diphtheroid spp. (table 47 ), and staph. aureus, enterbacteriaceae and candida spp. amounted to a total of 10% of isolates. 'Normal flora' were a mixed group of normal upper respiratory tract commensals and what might be considered innocuous flora accounted for over 80% of all isolates. Apart from all five isolates of candida spp. being in those who received TSN there was no difference between those receiving TSN and those receiving NEOCON.

Patients in the isolators receiving extensive and time consuming decontamination procedures were studied to determine if the microbial load had been markedly reduced by the regimen in table 41. Reduction in the microbial growth was assessed using this scoring scheme:

0 = no growth
1 = scanty growth (±)
2 = moderate growth (+)
3 = heavy growth (++ or +++)
(multiple organisms were counted separately).

The first 13 patients in the isolator achieved the reduction in average score shown in table 48 . The surfaces were reduced from a moderate growth to a very scanty growth and the orifices from a fairly heavy growth to an almost moderate growth. Stools remained more than moderately full of viable organisms. Females were more contaminated than males on admission but the same general degree of decontamination was achieved in both males and females. Males had more sterile specimens than females (table 46), so the non-sterile specimens from males were considerably more contaminated with organisms than the equivalent specimens from female children.

Discussion

The reduction in microbial load is very disappointing for the amount of effort entailed in administering the complicated regimen in table 41 and the low prevalence of pathogens as shown by these surveillance cultures makes the institution of any more radical cleansing regimen probably unrewarding. Other

Table 47

RESULTS OF SURVEILLANCE MICROBIOLOGY ON TRANSPLANTED CHILDREN (RMH)

| | surface (axillae, toes) | orifices (nose, throat, vagina) |
|---|---|---|
| No. of cultures | 82 | 101 |
| No. sterile | 11 (13%) | 9 (9%) |
| No. of isolates | 80 | 125 |
| Isolates of | | |
| Staph. aureus | 0 | 5 |
| Enterobacteriaceae | 3 | 8 |
| Candida sp. | 0 | 5 |
| Staph. epidermidis | 71 | 56 |
| "Normal flora" | 0 | 28 |
| Diphtheroids | 3 | 6 |

Table 48

## REDUCTION IN MICROBIAL LOAD BY DECONTAMINATION

average score/swab

| site | Pre-decontamination | on full decontamination |
|---|---|---|
| hairline | .5 | .29 |
| axillae | 1.8 | .52 |
| groins | 2.9 | .79 |
| umbilicus | 2 | .41 |
| mean | 1.8 | .5 |

| | | |
|---|---|---|
| nose | 1.61 | .13 |
| throat | 2.77 | 1.74 |
| mouth | 2.57 | 2.02 |
| vagina | 2.88 | 2.15 |
| mean | 2.46 | 1.51 |

| | | |
|---|---|---|
| stools | 3.85 | 2.76 |

| | | |
|---|---|---|
| (excluding stools) | | |
| males | 1.76 | .93 |
| females | 2.27 | .99 |

authors have attempted extensive decontamination of particular areas of the patient but without great success.

Bodey and Rosenbaum (1974) found 47% of skin organisms to be potential pathogens. The perianal area, groin and chest were the most contaminated; the back, abdomen, neck and scalp the least contaminated. Despite their skin cleansing regimen (tables 40 and 49 ) one-third of patients had persisting skin pathogens and 40% had persisting skin fungi. Despite topical antibiotics pathogens persisted in the groins in 25% of patients. Phisohex (3% hexachlorophane) was the best skin decontaminant of the three they used.

Their regimen resulted in 75% of skin cultures showing no aerobes and 50% of skin fungal cultures being sterile. Levitan and Perry (1967), Levine et al (1973) and Klastersky et al (1974) all found 70% of skin aerobic cultures were sterile with no antibiotics being used, only a daily hexachlorophane bath. This degree of skin sterility was achieved in the isolator children studied but not in the AML children whose cultured sites, the axillae and toe clefts, are much more likely to be contaminated than flat open areas of skin such as the back or abdomen.

Almost every study has shown the difficulty of decontaminating the oral cavity. About half the organisms isolated by Bodey and Rosenbaum (1974) were potential pathogens, mostly streptococci and though usually a two-log decrease was achieved, a quarter of the patients had persisting pathogens. The complexity of the oral regimens varied from that of Bodey and Rosenbaum (table 40 ) to that of Schimpff et al (1975) who used nothing additional to GVN which by itself gave a "reduced" oral flora. Buckner et al (1978) reported that of 12 patients who took all their GVN only 2 (17%) had continuous suppression of oral and nasal microbes. No study gives any consideration to the mechanical and dilutional role of repeated mouthwashes, which I believe to be very important.

The ears and nose are relatively harmless in microbiological content with the exception of nasal staph. aureus carried by 20% of the population (Bagshawe et al 1978). If present, this should be suppressed as transfer to other sites is common. Two of the nine AML children had nasal staph. aureus and one of these was the only AML transplant patient of 29 in the

Table 49

SKIN CULTURE RESULTS WITH DIFFERENT DECONTAMINATION REGIMENS IN AML

| Author | Skin regimen | Skin cultures positive | |
|---|---|---|---|
| | | for aerobes | for fungi |
| Levitan and Perry (1967) | Phisohex/halogenated soap alternate days | 25% | 65% |
| Dietrich et al (1973) | TEGO daily bath | 53% | |
| Levine et al (1973) | unstated but may be purchased! | 30% | 8% |
| Klastersky et al (1974) | hexachlorophane soap wash daily | 30% | |
| Bodey and Rosenbaum (1974) | Phisohex/halogenated soap + antibiotics to groins and perineum q.d.s. | 25% | 50% |

NEOCON/TSN study to develop a staph. aureus bacteraemia. Neomycin nystatin, polymixin and vancomycin ointment will eliminate 80% of aerobes from the nose, the same proportion as with much simpler methods e.g. Naseptin (0.5% neomycin in 0.1% chlorhexidine) cream.

The male genital mucosa is rarely a problem (except for herpes virus) and can be treated in the same way as the skin but the vagina harbours potential pathogens, usually the same as those in the stool. Measures adopted include chlorhexidine 1% obstetric cream, acetic acid 0.25% douches and multiple antibiotic gels inserted twice daily (Table 40 ). Some apparent decontamination will result, but irritation may often be caused.

Normal washing and hygiene remain very important and the use of expensive, potentially sensitising, sticky antibiotic gels is probably unnecessary, potentially harmful and their value has never been proved. The vagina, almost the most contaminated area (table 48 ), proved the most difficult to decontaminate in the Westminster children and in the Royal Marsden children this same area had almost twice as many isolates per swab than any other site sampled.

Our ability to suppress the micro-organisms of the body surface and orifices using the AFRACO gut regimen and a complicated series of skin and orifice decontamination techniques has not been very effective. However, body surfaces achieved greater sterility than body orifices. Infants with combined immune deficiency were easier to decontaminate than older children with aplastic anaemia. Females were more contaminated than males on admission but both achieved the same degree of residual bacterial growth following extensive decontamination. A complicated surface and orifice decontamination programme gave microbiological results that do not justify the time and effort even within a stringently protected environment. It is probably worthwhile suppressing nasal staph. aureus providing the protected environment is such that new acquisition will not occur. There is no reason to believe that sampling more sites more frequently will give more useful information.

## B) THE CLINICAL BONE MARROW TRANSPLANTATION OF THIRTY-ONE INFANTS AND CHILDREN

### Management of Children undergoing bone marrow transplantation

There are two aspects to this; the discussions necessary with the child, the family and the staff both before and during the transplant and the practical day to day medical programme throughout the hospital stay.

#### The necessary discussions

Unless an incompatible transplant is envisaged, tissue typing and mixed lymphocyte culture (MLC) will indicate whether the option of grafting is available. The question of whether or not to graft, and when, and with which donor can then be discussed. Though medical staff may recommend a marrow transplant, it must be the parents final decision whether or not to accept this advice.

The present results of conventional therapy and of grafting must be fully discussed, with frank admission of that which is not known. At least two separate discussions with the parents and hopefully the grandparents are necessary. Grandparents are a little removed from the situation and when kept informed may provide a useful shoulder for the parents despite family mobility reducing their role in recent years. The family need a concise, honest account of the procedure, and of the natural history of the illness stressing the difficulties, dangers and problems of each course of action. Such unwanted effects as hair loss and sterility, must be included in the discussion. I encourage parents to take notes during the discussion so they have information to fully discuss at home and on which to base further questions during a subsequent discussion. The difficulties of this discussion are least when the child's initial illness has been treated by those proposing to do the transplant. Conversations with the parents alone are necessary and a talk with the child and with the donor without the parents present will allow exploration of such fantasies as the children have about the procedure.

If either parent refuses to agree then careful thought must be given before proceeding with the transplant. Separated

or divorced parents should both be involved in the discussions and granting of permission. Should the parents so wish, they should speak to other parents and children who have had transplants. If a second opinion is requested then every effort must be made to obtain this from an appropriate specialist in another centre.

Talking to the child

Explaining the procedure to the child has proved remarkably easy. The concept of new blood or a new bone marrow is readily understood by children of a comprehending age. The two things which usually worry the children most are that they will lose their hair and that they will require repeated blood tests. The value of the long intravenous Hickman line allowing blood to be both given and taken is readily appreciated. It is particularly important to explain how the preconditioning will create a space for the new marrow and that the transplant is given through the intravenous line so holes do not have to be drilled in the bones to put the new transplant in place. Explanation of the isolation facility is necessary and a visit to this is almost mandatory. It is important that the degree of isolation has already been decided on,so that questions of the nature "Can mummy come in and talk to me?" can be answered positively at that time.

Five to six weeks, the usual in-patient stay, is an eternity to a young child so it is unwise to be specific about the duration of stay and confine the information to "until you are better". An elective transplant should not coincide with Christmas or a birthday if at all possible.

I do not consider it productive to describe all the possible complications to the child but the likely and 'simple' ones for which there is a good treatment may be mentioned, providing the treatment for these will be understood. The predictable and transient side-effects can all be described and a series of time goals set. A leisurely hour or two divided between several visits and involving casual but interested conversation about school, sport, friends and other interests is never wasted and will often reveal previously unexposed fears and misconceptions which can be corrected.

The parents during the transplant

Parents resident in the hospital may be a long way from home with socio-economic problems and guilty feelings about their lack of attention to their other children. Chronic anxiety and nightmares give way to periods of depression and elation. These spells do not coincide in each parent so each does not get the required response from the other. They may feel displaced from their traditional role by a technology with which they only slowly come to terms. After about two weeks they play an increasingly constructive role with apparently less labile emotions, taking a more active part in the ward and helping more with their child. Although it is helpful to have the parents actively involved they must also be sure to get away from the hospital from time to time. They become friendlier and more informal with the staff but may still be occasionally hostile and impatient, particularly if the transplant is not proceeding uneventfully.

It is better that parents, together, get information from only one or two people. Some ask everyone's opinion about every aspect and, because they hear only part of half-factual replies, an ideal situation for misunderstandings easily arises. However often one parent will want to talk separately from the other and this must be appreciated. As always, it is infinitely easier to tell the truth, but with an optimistic air. Even when the child is well, the predictable physical effects of various treatments should be stressed.

The staff during the transplant

An emotional load also falls on the staff. The same nurse should not always look after the same patient but if this is unavoidable careful watch needs to be kept for signs of nervous fatigue. Whilst no-one wishes staff uncaring for their patients, at the same time they must be able to stand back emotionally from the situation. The children need caring staff, not more parents. Some transplant units have daily meetings where staff can ventilate their feelings but this public forum does not suit everyone. A resident hospital chaplain may be of inestimable help to both the staff and also the parents whether or not of a religious persuasion.

A short time should be available each day when those responsible for the transplant programme are available to any one of the many different disciplines involved. Twice weekly more formal meetings of no more than one hour at which any involved person can be present are also helpful. These allow the non-medical staff to be kept informed of the patients' progress and why certain decisions are being taken. Meetings like these also allow relevant staff to participate in the reaching of decisions and the staff can be kept informed of previous patients' progress now they are at home. Every three months a meeting should be given over to the nurses to air their complaints and frustrations. Taking nurses or ancillary professional staff to outside meetings is good for morale and encouraging the writing of articles makes then crystallise their thoughts and attitudes.

The childhood donor

The child donor should be off school for 5 days before the transplant, and longer if susceptible to prevalent measles or chickenpox. The donor should be kept away from the common cold, not so much because of the risk of virus transmission in the transplant but to avoid the addition of any unnecessary risk to the donor who usually has a general anaesthesia for the marrow harvest. Spinal anaesthesia would be less than ideal in a young child. The donor should be assessed for fitness at an early stage in the proceedings by a physician independent of the transplant team, and should have a blood count, liver function tests and urinalysis. Routine chest x-ray and electrocardiogram are unnecessary. Both parents should give their permission as should the donor if able to understand the procedure. The legal aspects of this permission have never been tested in the courts of the United Kingdom but this will happen, probably with a donor in the care of a local authority. Harm to the donor has not yet been reported, and the situation is quite different from that of a living kidney donor whose donated organ is not self-replacing. A pregnant donor should not be used.

It is usual to venesect 5-10 mls/kg from the donor a week

before the transplant. Some protocols require the white cells so obtained to be given to the patient and the red cells to be available for return to the donor after the marrow extraction. This venesection may stimulate the donor marrow and give a more cellular yield. The return of the autologous red cells will reduce the transient hypovolaemia following marrow harvest and avoids using allogeneic blood. The donor is usually discharged the next day and should be given iron supplements for six weeks. Post-graft donor leukaphoreses (occasionally recommended) will put a severe strain on a young donor, but may be possible on a 'spin-and-return' basis.

The day to day medical programme

A check list of investigations is essential as the programme is too complicated to be readily remembered each day. A routine list also ensures comparability of follow-up, allowing post transplant patterns to be identified. The list, which combines routine and research procedures, requires regular review. Infants and toddlers will need a modified programme, and some investigations are only applicable to certain diagnoses.

Pre-transplant

Pre-transplant investigations (table 50) fall into a number of sections and do not include investigations into the nature of the disease. Those with asterisks also apply to the donor. From table 50 a number of points should be noted.

Hepatic activation of cyclophosphamide may be compromised by previous medication and since occult hepatitis is most common among young children both donor and recipient may be at risk. The metabolism and clearance of drugs will be affected by poor renal function and good glomerular function is necessary during the diuresis required with cyclophosphamide preconditioning. Serum amylase and CPK levels rise after cyclophosphamide or TBI to levels of 5000 units and 400 units respectively but both should be normal by 3-4 days later. If the cerebrospinal fluid is abnormal or the marrow contains more than 5% blasts, the transplant should probably be postponed.

Surveillance cultures with antibiotic sensitivities are especially important if the child has been transferred from

Table 50

## PRE-TRANSPLANT PROCEDURES

### Haematology

*Full blood count, platelets, reticulocytes
Haemoglobin F
Bone marrow
Clotting screen
Cerebrospinal fluid cytology

### Biochemistry

Urea and electrolytes
*Bilirubin, liver enzymes and alkaline phosphatase
Uric acid
Calcium, phosphate
Amylase
Creatinine and creatinine clearance
Creatine phosphokinase (CPK)
*store 20 mls serum and 20 mls plasma

### Microbiology

Surveillance cultures
Stool culture and ?preservation of certain cultures
Microscopy of urine for inclusion bodies and viruses

*Antibody titres

| | |
|---|---|
| Hepatitis A and B | Toxoplasma |
| Cytomegalovirus | Herpes simplex |
| Varicella | Rubella |
| Measles | EB virus |
| Mumps | Pneumocystis |
| Polyoma virus (JC/BK) | |

### Immunology

*T and B lymphocyte sub-populations
*T lymphocyte function (PHA, MLC, candida responses)
*Immunoglobulins
*Specific antibody responses (Tetanus, Salk Polio, isohaemagglutinins)
*Salivary IgA and free secretory piece
*Schick test
*Delayed hypersensitivity to SK-SD, PPD, Mumps, Candida
*Enzymes associated with immune deficiency

Table 50 (contd.)

Histocompatibility laboratory

*Tissue typing of family

*Mixed lymphocyte cultures against family (MLC)

*Cross-matching against donor

*Red cell isoenzymes and genotyping

* $G_m$ and $K_m$ allotyping

Storage of more than $5 \times 10^6$ lymphocytes

Chromosomes ± banding

Radiology

Chest

Sinuses

Wrists and hands

Additional procedures

*Photograph

Respiratory Function Tests

Electrocardiogram

?Pneumococcal immunisation

Sperm store

another hospital. Antibiograms to choose an appropriate decontamination regimen are used in some centres but are of little practical value (EORTC 1980). In-vitro preservation of stool flora gives the option of full autologous recontamination. Specific antibody titres indicate whether certain infections have definitely occurred in both the donor and recipient. A rise in a donor's antibody titre a few days after the graft increases the chances of having transferred that organism during the transplant. The viruses thought important which may be transferred during the transplant are cytomegalovirus and EB virus but recipient antibodies should provide a degree of protection. There is little point in seeking CMV or EBV antibody negative blood product donors, if the recipient or donor were antibody positive before the graft.

The immunological investigations are most relevant to transplants for immune deficiency where a number of other research investigations may be appropriate. Donors, possible heterozygotes, should have the same investigations. The documentation of simple immunological recovery in non-immunodeficiency diseases is interesting but at present does not have great practical value. However transplant regimens change and the monitoring of immunological recovery using increasingly sophisticated immunological tools may be valuable in understanding immunological ontogeny and the early prediction of graft-versus-host disease.

The histocompatibility investigations relate to finding the donor and documenting engraftment. Red cell grouping, erythrocyte isoenzyme and genotyping studies will prove red cell engraftment and $K_m$ and $G_m$ allotyping may reveal the source of subsequent immunoglobulin production. Chromosome banding studies may document engraftment when other markers are not available. Storage of lymphocytes is important in an aplastic patient. Should the graft fail to take and the patient thereafter have severe leukopenia, there will be no way of matching an alternative donor unless lymphocytes are stored. Those stored may not be effective responders in the MLC but should stimulate adequately in predicting GVHD.

Depending on the urgency of the transplant, radiologically opaque sinuses should be treated before immunosuppression. This is more important (and more difficult) in aplastic patients than

leukaemia patients. Wrist and hand x-rays are included both for morphological and future auxometric studies. Hormonal studies may be thought worthwhile. Respiratory function tests provide a base line and serial repetition may identify those who will develop interstitial pneumonitis although almost all patients with acute myeloid leukaemia have impaired lung function pre-transplant (Barrett and Depledge 1981). Sequential electo-cardiography is necessary during 4 day cyclophosphamide conditioning but will not indicate anthracycline toxicity in the leukaemic children.

Pneumococcal immunisation (Pneumovax), though unproven in this country, is effective in preventing pneumococcal septicaemia in children with sickle cell disease (Ammann et al 1977). Pneumococcal infections are common in transplant recipients (Winston et al 1979) so despite an impaired response, pneumococcal immunisation should probably be given. Any child receiving total nodal irradiation (which includes the spleen) should be immunised and all transplant recipients remain on prophylactic penicillin until one year after the transplant.

Sperm storage may be applicable to a small number of children undergoing transplantation. Leukaemic males are subfertile even before anti-leukaemic chemotherapy and so storage of adequate sperm may be difficult. Cyclophosphamide preconditioning before transplantation for severe aplasia may not lead to sterility but the possibility of sperm cryopreservation should be considered. This whole matter needs handling with tact.

A number of other departments in the hospital need to be involved.

| | |
|---|---|
| Social services | Physiotherapists |
| Dental department | Ward teacher |
| Wigmaker | Chaplain |
| Dieticians | Psychiatrist |

A specific person in each department is informed about the child and the family. They should meet the parents and child to identify any specific help required and by liaising with the referring hospital and local authority integrate such help as is required, both during the admission and when the child returns home.

If possible, dental problems should be resolved before

the trarsplant and the continuing interest of a paediatric dentist leads to a high standard of mouth hygiene. The dieticians' role has been previously discussed. A resident hospital chaplain, available at all times, provides excellent support for parents and staff whether or not they have religious beliefs. For more formal support and discussion the psychiatric department is available. Though some parents find this helpful, many have preconceived ideas of the role of the psychiatrist. However, there is great value in psychiatric advice when the staff are uncertain how to handle a particular circumstance.

The in-patient programme

My present in-patient programme for marrow transplantation at the Royal Marsden Hospital is shown in table 51 . This is for children with AML, but would be the same in principle for those with ALL, or aplastic anaemia. The transplant is given the day after the irradiation for purely local reasons. All children are transplanted in single cubicles with positive pressure filtered air. For children with aplasia the preparation would be somewhat different. Usually four days of cyclophosphamide, (1.5 gms/$m^2$/day) are given but more complicated regimens may also be used.

The isolators used at Westminster Children's Hospital will require the initial timetable to be different but the differences are trivial. With isolators the programme would start four days earlier with decontamination. If microbiological surveillance cultures on days -10 and -8 were satisfactory when read on day -7 the child would have the theatre procedures on day -6 and enter the isolator on return from theatre. If there were reasons for delay, the child could enter one isolator until decontamination was acceptable, go to theatre from there, and return to a second isolator. TBI can be and has been performed in a transit isolator for one child already extremely well decontaminated and in an isolator. More usually a child with leukaemia would enter the isolator on return from TBI.

High dose cyclophosphamide may cause haemorrhagic cystitis, myocardial failure and fluid retention. A good urine flow is essential and adults with leukaemia will have urinary catheters inserted. Their neutrophil counts are normal, unlike the aplastic

Table 51

## PROTOCOL FOR MARROW TRANSPLANT PATIENTS

(pre-transplant)

| Day of week | Day | |
|---|---|---|
| Mon | -9 | Admission. CXR, ECG, Radiotherapy planning, Lung function tests |
| Tues | -8 | Start antifungal agents. Stool + all screening swabs |
| Wed | -7 | Urinary Catheter. Hickman line, Bone marrow + L.P. + I/T Cytosine 40 mg/$m^2$, chromosomes and markers. |
| Thurs | -6 | Leukaphoresis. Start antibacterial agents 1800 hrs. Virology screening specimens. Stool. |
| Fri | -5 | Venesect 1 pint from DONOR. FBC, U&E, LFTS, Amylase |
| Sat | -4 | Cyclophosphamide 1.8 gms/$m^2$ + phenobarb 60 mg/$m^2$ + chlorpromazine 25 mg<br>ECG @ 6 hrs. FBC, U&E |
| Sun | -3 | Cyclophosphamide 1.8 gms/$m^2$ + phenobarb 60 mg/$m^2$ + chlorpromazine 25 mg<br>ECG pre-cyclo FBC, U&E |
| Mon | -2 | Premed @ 2200 hrs phenobarb. 60 mg/$m^2$ + dexamethasone 8 mg po Screening swabs and Stool FBC U&E |
| Tues | -1 | 0700 Phenobarbitone 60 mg/$m^2$ IV + dexamethasone 8 mg IV TBI<br>Start Cyclosporin 6.25 mg/kg bd IM |
| Wed | 0 | Bone marrow transplant |

patients in whom a transient bacteraemia is much more serious. Catheterising a child is an unwarranted interference. An appropriate diuresis programme is detailed below

DIURESIS WITH HIGH DOSE CYCLOPHOSPHAMIDE

| | |
|---|---|
| Start | 1 hour before cyclophosphamide |
| Rate | 250 mls/$m^2$/hour for 5 hours<br>200 mls/$m^2$/ hour for 4 hours<br>100 mls/$m^2$/hour for 15 hours<br>3550 mls/$m^2$/day over 24 hours |
| Fluids | Dextrose/Saline 500 mls alternating with<br>N/2 saline 500 mls |
| Additions | At least 10 mmols $K^+$/500 mls fluid |
| Drugs | Frusemide 20 mg/$m^2$ at 1 hour and 6 hours<br>Promethazine 35 mg/$m^2$ before and 4 hourly prn |
| Check | Sodium and potassium daily and after 8 hours during courses one and two, ECG before cyclophosphamide and after 6 hours during each course |

This supplies about 180 mmols of sodium/$m^2$ and at least 75 mmols of potassium per square metre. Twice this amount of potassium may be necessary. If gentamicin and carbenicillin are being administered concurrently with cyclophosphamide, normal saline and even more potassium will be required. Chlorpromazine 10 mg/$m^2$ will also be required if there is vomiting. The intention is that the child should sleep for the first 8-10 hours. A platelet count of more than 50,000/µl is required to prevent intracranial haemorrhage in the event of vomiting. In aplasia, the transplant is given 36 hours after the last dose of cyclophosphamide.

Doses are quoted per metre squared and not per kilogram. A certain dose/kg will be the same amount /$m^2$ for a nine year old but if given /kg to a two year old will only be 75% of dose/$m^2$ and an infant of 3.5 kg would only receive 40% of the equivalent dose/$m^2$. Teenagers will be relatively overdosed if based on mg per kg.

One aplastic child had been intermittently septicaemic, with profuse diarrhoea and normal plasma biochemistry. She died suddenly the day before her transplant and was the only patient with a drop in the electrocardiogram voltage (table 52 ). This change was only in the limb leads and no other patient showed more than 10% reduction in ECG voltage. No autopsy was allowed so there was no specific evidence that cyclophosphamide caused her death, but should this voltage drop occur, the regimen of cyclophosphamide will need adjusting.

Table 52

Average voltage/standard

during four cyclophosphamide courses

| | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|
| | PRE | +6hrs | PRE | +6hrs | PRE | +6hrs | PRE | +6hrs |
| chest leads | 6.6 | 6.1 | NE | 6.1 | 6.3 | 6.5 | 5.4 | - |
| limb leads | 11.4 | NE | 11.8 | 12.5 | 12 | 12.7 | 7.4 | - |

NE - not evaluable

Investigations after grafting

Routine investigations after the graft are shown in table 53. An additional marrow examination on day 21 is justified if there is no peripheral reconstitution. If this aspirate is 'empty' immediate regrafting using the same donor but with either no or different preconditioning is indicated. By the time this is organised slow reconstitution may have appeared. Should a second graft fail and leave the patient profoundly aplastic, either stop treatment or use the stored lymphocytes in an attempt to find another donor. The outlook in this situation is grave.

Methotrexate is given post-graft as GVHD prophylaxis unless cyclosporin-A has been commenced pregraft. The usual intravenous regimen is 15 mg/$m^2$ on day 1, followed by 10 mg/$m^2$ on days 3, 6, 11 and weekly thereafter to day 102. With poor marrow recovery, omit the day 18 dose. If hepatic or renal function is impaired, give folinic acid (3 mg/$m^2$) 12 and 24 hours after each dose.

Blood products must be irradiated to 1500 rads and chronic granulocytic leukaemia (CGL) cells to 5000 rads for one year after the transplant. This radiation does not impair neutrophil function (Van der Meer 1980) but will prevent stem cells in the

# POST GRAFT INVESTIGATIONS FOR IN-PATIENTS

Table 53

| | | Day. vol | W 0 | T +1 | F +2 | S +3 | S 4 | M 5 | T 6 | W 7 | T 8 | F 9 | S 10 | S 11 | M 12 | T 13 | W 14 | T 15 | F 16 | S 17 | S 18 | M 19 | T 20 | W 21 | T 22 | F 23 | S 24 | S 25 | M 26 | T 27 | W 28 | T 29 | F 30 | S 31 | S 32 | M 33 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Blood | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| EDTA (pink) | FBC | 2.5 | ✓ | ✓ | | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ |
| Li Hep (orange) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | U&E | 7 | ✓ | ✓ | | ✓ | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ |
| | Creatinine | | | | | | | ✓ | | | | | | | ✓ | | | | | | | ✓ | | | | | | | ✓ | | | | | | | ✓ |
| | ALT/AST | | | ✓ | | | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ |
| | γGT/APhos | | | ✓ | | | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ |
| | Bilirubin | | | ✓ | | | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ |
| | Ca $PO_4$ Mg | | | | | | | ✓ | | | | | | | ✓ | | | | | | | ✓ | | | | | | | ✓ | | | | | | | |
| | Cholesterol | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | ✓ | | | | | |
| | Amylase | | | ✓ | | | | ✓ | | | | | | | ✓ | | | | | | | | | | | | | | ✓ | | | | | | | |
| PFH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Chromosomes | 10 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | ✓ | | | | |
| | T & B markers | 10 | | | | | | | | | | | | | | | | | | | | | | | | | | | ✓ | | | | | | | ✓ |
| Citrated Special | Clotting studies | 4.5 | ✓ | | | | | ✓ | | | ✓ | | | | ✓ | | | ✓ | | | | ✓ | | | ✓ | | | | ✓ | | | | | | | ✓ |
| | FDP | 2.5 | ✓ | | | | | ✓ | | | ✓ | | | | ✓ | | | ✓ | | | | ✓ | | | ✓ | | | | ✓ | | | | | | | ✓ |
| | | | 0 | +1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 |
| Clotted (plain) | Alb/Glob | 2 | | ✓ | | | | ✓ | | | | ✓ | | | | | ✓ | | | | | ✓ | | | | ✓ | | | | ✓ | | | | | | |
| | Store serum | 10 | | | | | | ✓ | | ✓ | | ✓ | | | ✓ | | | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ |
| | DCT | 2 | | | | | | | | ✓ | | | | | | | | | | | | | | ✓ | | | | | | | | | | | | |
| | Immunoglobulins +E | 3 | | | | | | | | | | | | | ✓ | | | | | | | | | | | | | | ✓ | | | | | | | |
| | Isohaemagglutinins | 2 | | | | | | | | | | | | | ✓ | | | | | | | | | | | | | | ✓ | | | | | | | ✓ |
| | Serum folate | 2 | | | | | | | | | ✓ | | | | | | | | | | | | | | | | | | | | | | ✓ | | | |
| | B1 group Ag | 2 | | | | | | | | | | | | | | | ✓ | | | | | | | | | | | | | | | | ✓ | | | |
| | Virology serum | 5 | | (see below) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Gentamicin levels | 2 | | | | | | | | | | | | | | | | | 48–72 hours after starting Gentamicin | | | | | | | | | | | | | | | | | |
| Marrow | + markers | | | | | | | | | | | | | | | ✓ | | | | | | | | | | | | | | ✓ | | | | | |
| | chromosomes | 2 | | | | | | | | | | | | | | ✓ | | | | | | | | | | | | | | ✓ | | | | | |
| | | | 0 | +1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 |
| Microbiology | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Urine for BK | 50 | ✓ | | | | | | | ✓ | | | | | | | ✓ | | | | | | | ✓ | | | | | | | ✓ | | | | | |
| | Urine to Hammersmith | 25 | | ✓ | | | | | | | ✓ | | | | | | | ✓ | | | | | | | ✓ | | | | | | | ✓ | | | | |
| | Mouth swab (virology) | | | ✓ | | | | | | | ✓ | | | | | | | ✓ | | | | | | | ✓ | | | | | | | ✓ | | | | |
| | screening swabs (ie N/T.Ax. Toes Vagina) | | | | | | | ✓ | | | | | | | ✓ | | | | | | | ✓ | | | | | | | ✓ | | | | | | | ✓ |
| | stool | | | ✓ | | | | ✓ | | | ✓ | | | | ✓ | | | ✓ | | | | ✓ | | | ✓ | | | | ✓ | | | ✓ | | | | ✓ |
| | Serum (H'smith) | 5 | | ✓ | | | | | | | ✓ | | | | | | | ✓ | | | | | | | ✓ | | | | | | | ✓ | | | | |
| Other studies | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Albumin study | | | | | | | | | | | | | | | | | | ⟷ | | | | | | ⟷ | | | | | | | | | | | |
| | Lung function tests | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | ✓ | | | | | |
| | Cyclosporin levels (serum) | 10 | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | | ✓ | | | ✓ |
| | Vitamin bloods | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | ✓ | | | |
| | | | 0 | +1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 |

JGW
RMH

↑ change cyclosporin A to 6.25 mg/kg bd po

At discharge VIGG 750 mg
start Septrin tabs T bd
Folic acid 5 mg daily

transfusion being able to proliferate and establish an incompatible clone with the risk of GVHD.

For infants with SCID the programme in table 53 must be curtailed. They do not usually require preconditioning and so avoid all the related investigations. However, they often require intravenous feeding with appropriate surveillance. Laboratory micro-methods should allow adequate monitoring with about 25 mls of blood per week. Even so this is 9% of the blood volume of a 4 kg infant so irradiated transfusions will be required every 4 weeks.

After discharge home

An out-patient follow-up programme is shown in table 54 . This combines research and routine investigations and must be reconsidered from time to time. As 60 mls of blood are requested some weeks it needs to be reviewed for younger children with considerable inter-laboratory liaison to ensure parts of each specimen are not wasted.

Haemopoiesis and evidence of engraftment clearly need to be documented and close watch kept on renal and hepatic function. Immunological reconstitution in leukaemic and aplastic patients has been well studied but the use of cyclosporin-A adds a new dimension. The virology studies are part of an on-going investigation.

On leaving protective isolation en route directly for home, children should receive 10 mg/kg of varicella immune globulin (VIG) and 5 mg/kg every two weeks until 14 weeks after grafting. Acyclovir does not make this precaution obsolete. They should receive Septrin for 6 months as prophylaxis against pneumocystis carinii. Penicillin-V should be given from when Septrin is stopped until 1 year after grafting. Oral antifungal agents may be required for three months.

They and their parents need instruction about avoiding infection hazards. This is best done verbally but also with a sheet of simple guidelines, (table 55 )most of which are commonsense. They must realise the dangers of measles and chickenpox. Avoiding direct sunlight only applies to those who have had irradiation. Just how necessary all these precautions are is difficult to prove and it is likely they can be somewhat

Table 54

MARROW GRAFTS - POST GRAFT INVESTIGATIONS (OUT-PATIENTS)

| | | | Week | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 26 | 30 | 34 | 38 | 42 | 52 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Month | | | | | 2 | | | | 3 | | | 4 | | | 6 | | | 8 | | 10 | 12 |
| | | vol | | | | | | | | | | | | | | | | | | | | | | |
| **Blood** | | | | | | | | | | | | | | | | | | | | | | | | |
| EDTA. (pink) | FBC | 1 | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| Lithium Heparin (orange) | | | | | | | | | | | | | | | | | | | | | | | | |
| | U&E | 7 | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| | Creatinine | | | ✓ | | ✓ | | ✓ | | ✓ | | ✓ | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | |
| | ALT/AST | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| | γGT/APhos | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| | Bilirubin | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| | Cholesterol | | | | | ✓ | | | | | | ✓ | | | ✓ | | | | ✓ | | | | | |
| PFH | | | | | | | | | | | | | | | | | | | | | | | | |
| | Chromosomes | 10 | | | | | | | ✓ | | | | ✓ | | | | | | ✓ | | | | | ✓ |
| | T & B markers | 10 | | ✓ | ✓ | ✓ | | ✓ | | ✓ | | ✓ | | ✓ | | ✓ | | | ✓ | | | | | |
| Clotted | Alb/Globulin | 2 | | | | ✓ | | | ✓ | | | ✓ | | | ✓ | | | | ✓ | | | | | |
| | store serum | 10 | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| | Immunoglobulins + E | 3 | | | ✓ | | | ✓ | | | | ✓ | | | ✓ | | | | ✓ | | | | | ✓ |
| | DCT | 2 | | | | ✓ | | | | | | ✓ | | | | | | | | | | | | |
| | Isohaemagglutinins | 2 | | ✓ | ✓ | ✓ | | ✓ | | ✓ | | ✓ | | ✓ | | ✓ | | | ✓ | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | |
| | Bl. gp. antigen | 2 | | ✓ | | | | | ✓ | | | | ✓ | | | | | | ✓ | | | | | ✓ |
| | Virology serum | 5 | | | (see below) | | | | | | | | | | | | | | | | | | | |
| | | | | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 26 | 30 | 34 | 38 | 10mths | 12 |
| **Marrow** | + markers | | | | | | | | ✓ | | | | ✓ | | | | | | ✓ | | | | | |
| | + chromosomes | | | | | | | | ✓ | | | | ✓ | | | | | | ✓ | | | | | |
| **Microbiology** | Virology cells (vide supra) | | | ✓ | ✓ | ✓ | ✓ | ✓ | | | | ✓ | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | ✓ |
| | Urine to Hammersmith | 25 | | ✓ | ✓ | ✓ | ✓ | ✓ | | | | ✓ | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | ✓ |
| | mouth swab (viral) | | | ✓ | ✓ | ✓ | ✓ | ✓ | | | | ✓ | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | ✓ |
| | serum to Hammersmith | 5 | | ✓ | ✓ | ✓ | ✓ | ✓ | | | | ✓ | | ✓ | | ✓ | | ✓ | | | ✓ | | ✓ | ✓ |
| OTHER STUDIES | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | |
| | Lung function tests | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | | ✓ | | ✓ | | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| | Cyclosporin levels (serum) | 10 | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | | ✓ | ✓ |
| OTHER TREATMENTS | | | | | | | | | | | | | | | | | | | | | | | | |
| | VIGG 500 mg | | | ✓ | | ✓ | | ✓ | | ✓ | | ✓ | ✓ | | | | | | | | | | | |
| | Septrin tabs T bd | | | continue until 6/52 after stopping cyclosporin or for at least 6/12 | | | | | | | | | | | | | | | | | | | | |
| | Folate 5 mg daily | | | continue until 6/52 after stopping cyclosporin or for at least 6/12 | | | | | | | | | | | | | | | | | | | | |
| | | | | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 26 | 30 | 34 | 38 | 10 | 12 |

Table 55

PRECAUTIONS TO TAKE AFTER A BONE MARROW GRAFT

Risk of infection at home will be reduced to a minimum by the following procedures, which you should follow for five months after going home.

a) If anybody in the family has a cold, they should keep away from you.

b) Pets should not be allowed to lick you, and you should get no new pets.

c) Visitors should avoid touching you, and should not come if they have a cold, influenza, diarrhoea or a boil.

d) Members of the family at school or work should stay away if there is an epidemic of chickenpox or measles. If you come in contact with one of these infectious diseases get in touch with us immediately.

There is no need to feel tied to the house; fresh air is beneficial, but stick to the following rules:-

i) Don't go into crowded shops or cinemas, cafes and discos.

ii) Go into the countryside rather than the town for excursions, but avoid direct sunlight if possible during the first three months.

iii) Avoid crowded public transport.

Food

Eat freshly cooked foods and only salads which are freshly picked, home grown and well washed. Avoid take-away foods.

You may discontinue these precautions after five months.

JGW

reduced. Children will return to school 4-5 months after their transplant, or after stopping cyclosporin-A.

Infants transplanted for SCID have greater immune suppression for much longer. Consequently they need protection and a high standard of family hygiene for a year. A considerable level of medical awareness is required even later as they contract the common infections of childhood. Routine VIG is not necessary unless other children in the family constitute a risk but should other children be likely to bring chickenpox or measles home, appropriate immunoglobulin must be given. All killed immunisations should be given including Salk polio and pertussis, but should they be exposed to pertussis under the age of five, erythromycin prophylaxis should be employed.

The programme and schedules described do not cover all aspects of a transplant, but they have proved effective in my experience.

## SEVERE COMBINED IMMUNE DEFICIENCY IN INFANTS

The condition now termed severe combined immune deficiency (SCID) was previously called Swiss-type agammaglobulinaemia, a term derived from the descriptions of congenital agammaglobulinaemia by Bruton (1952) and "familial essential lymphocytophthisis" by Glanzmann and Riniker (1950). SCID is probably more common than is realised, but whether it accounts for 10% of the post-neonatal mortality as suggested by Rosen (1972) is doubtful. If so, over 300 British infants each year would be affected. Berry and Thompson (1968) found 14 of 1000 consecutive autopsies at Great Ormond Street Hospital to satisfy their diagnostic criteria for SCID of which half had been recognised as immune deficient during life. This, given the nature of in-patient population at that hospital, would suggest around 50 new cases occur each year in the United Kingdom.

By 1979 ten infants with SCID had been admitted to Westminster Children's Hospital for bone marrow transplantation or other appropriate therapy. In addition a seven year old male in Texas Children's Hospital Houston, Texas has also been in my care, giving an experience of eleven children and infants with SCID.

### Pre-transplant studies

### Initial presentation

Table 56 shows why the infants with SCID first presented to hospital. Candidiasis and respiratory infections were the most common presentations which is similar to the presentations in the report by Hitzig et al (1968) who found that 89% of these children had pneumonia; 85% diarrhoea; 79% candidiasis and 57% had cutaneous sepsis. Lung disease or diarrhoea were the most common problems in sixty-nine infants who ultimately received marrow transplants (Bortin and Rimm 1977), so the combination of respiratory infection, diarrhoea and candidiasis are important diagnostic pointers.

The mean age at first presentation was 13 weeks (range 3-18 weeks) and there were 4 females and 7 males. It was not possible to know in most cases whether the inheritance was sex-linked, autosomal recessive or sporadic. Traditionally there are supposed to be three times as many male infants with SCID

Table 56 (a)

REASONS FOR INITIAL REFERRAL (EXCLUDING DV*)

| | |
|---|---|
| Respiratory infection | 60% |
| Candidiasis | 60% |
| Diarrhoea | 30% |
| Failure to thrive | 30% |
| Other sepsis | 20% |

*DV - germfree delivery in Houston, Texas

Table 56 (b)

AGE AND PROBLEMS AT INITIAL HOSPITAL PRESENTATION

| Patient | Sex | age in weeks | Problems |
|---|---|---|---|
| LS | F | 3 | Respiratory infection, esch. coli septicaemia |
| PL | M | 5 | Diarrhoea, respiratory infection |
| HN | F | 9 | Oral candidiasis |
| SI | M | 13 | Oral candidiasis, respiratory infection |
| MP | M | 13 | Oral candidiasis, respiratory infection |
| GS | M | 14 | Respiratory infection, failure to thrive, candidiasis |
| TD | F | 15 | Diarrhoea, skin sepsis, failure to thrive |
| AJ | M | 16 | Oral candidiasis |
| DF | F | 18 | Oral candida, respiratory infection, diarrhoea |
| JWh | M | 18 | Failure to thrive |
| DV* | M | at birth | none |

*axenic delivery in Houston, Texas

compared to female infants and this was so in the series of 69 infants reported by Bortin and Rimm (1977).

Time to diagnosis

Table 57 shows the time elapsed between first presentation and full diagnosis. The mean interval to full diagnosis (at a mean age of 21 weeks) was 8 weeks. Only 1 (10%) had not developed considerable diarrhoea and two infants (at the time undiagnosed) had been mistakenly admitted to infectious disease hospitals because their diarrhoea was such that a severe bowel infection was suspected. The infant in Houston, maintained in a gnotobiotic environment, did not develop diarrhoea and thrived. This interval of 8 weeks to full diagnosis could be improved on but only to the extent of a week or two, unless profound lymphopenia was recognised on the initial admission. This finding, like many of the immunological features, is very variable despite the ultimate similarity of the clinical picture. Half the infants required a third hospital admission before diagnosis and only one was diagnosed on his first admission to hospital.

Immunological studies

Lymphocyte numbers

The International Bone Marrow Transplant Registry data (Bortin and Rimm 1977) described 69 infants with SCID who received haemopoietic tissue transplants. Lymphocyte counts were subnormal in 96% and half the infants had less than $1 \times 10^9$ lymphocytes per litre. Table 58 shows the lymphocyte and lymphocyte sub-population numbers in ten infants at transfer to Westminster Children's Hospital. All lymphocyte counts were subnormal and 80% had less than $1 \times 10^9$ lymphocytes per litre. T lymphocyte numbers were very low (mean $0.01 \times 10^9$/l) and could not be increased with in-vitro thymosin, the usual finding in SCID. Of 15 cases of SCID reported by Lewis et al (1977), half had less than $1 \times 10^9$ lymphocytes/litre with a mean of $0.09 \times 10^9$/litre (range 0-$0.38 \times 10^9$/l). Lewis et al (1977) found B lymphocyte numbers to be more variable and were sometimes normal, as in 62% of the Westminster Children.

In eight infants adequate data was obtained from the

Table 57

SEVERE COMBINED IMMUNE DEFICIENCY

TIME BETWEEN FIRST PRESENTATION AND DIAGNOSIS

| Patient | Age at first presentation (weeks) | Age at diagnosis (weeks) | Number of hospital admissions | Time to diagnosis (weeks) | Major intercurrent problems |
|---|---|---|---|---|---|
| LS* | 3 | 7 | 2 | 4 | continued diarrhoea (rotavirus) |
| PL | 5 | 18 | 3 | 13 | Diarrhoea, respiratory infections |
| HN | 9 | 20 | 2 | 11 | Diarrhoea, pneumonia x 2 |
| SI* | 13 | 24 | 3 | 11 | Diarrhoea, failure to thrive, pneumonia |
| MP | 13 | 20 | 2 | 7 | Diarrhoea, candidiasis |
| GS | 14 | 18 | 1 | 4 | Diarrhoea, candidiasis |
| TD | 15 | 17 | 2 | 2 | Diarrhoea, skin sepsis |
| AJ | 16 | 39 | 3 | 23 | Respiratory infections, fevers, failure to thrive |
| DF | 18 | 24 | 3 | 6 | Diarrhoea, candidiasis, respiratory infections |
| JWh | 18 | 24 | 3 | 6 | Diarrhoea, respiratory infections |
| DV** | at birth | 2 | - | 2 | None |

* previous child died of infection at less than 6 months of age

** previous child died of SCID

Table 58

## HETEROGENEITY OF SCID

### Lymphocyte numbers

| Patient | Age at transfer (in weeks) | Leukocytes $\times 10^9/l$ | Lymphocytes | |
|---|---|---|---|---|
| | | | $\times 10^9/l$ | as % of leukocytes |
| TD | 16 | 4 | 0.6 | 15% |
| LS | 16 | 12 | 0.24 | 2% |
| GS | 18 | 4.5 | 0.23 | 4% |
| MP | 22 | 4.8 | 1.6 | 30% |
| JWh | 25 | 7.8 | 1 | 13% |
| HN | 25 | 9.6 | 0.29 | 3% |
| DF | 37 | 5.3 | 0.53 | 10% |
| SI | 37 | 6.2 | 0.5 | 8% |
| AJ | 50 | 4.5 | 0.16 | 4% |
| PL | 70 | 6 | 0.8 | 13% |
| DV | 26 | 4.8 | 1.7 | 35% |

### Lymphocyte subpopulation numbers

| Patient | Lymphocyte subpopulations $\times 10^9/l$ | | | |
|---|---|---|---|---|
| | SRBCR 'T' lymphocytes | | Surface Ig bearing 'B' lymphocytes | |
| | patient | NR | patient | NR |
| TD | NA | | NA | |
| LS | 0.019 | 0.07–0.3 | 0.116 | 0.1–1.0 |
| GS | 0 | | 0.2 | |
| MP | NA | | NA | |
| JWh | 0 | | 0.12 | |
| HN | 0.014 | 0.08–0.7 | 0.015 | |
| DF | 0.005 | | 0.01 | |
| SI | 0.025 | 0.18–1 | 0.405 | |
| AJ | 0.028 | | 0.01 | |
| PL | 0 | 0.48–1.1 | 0.8 | |
| DV | NA | | NA | |

NR – normal range for age  SRBCR – sheep red blood cell rosettes

NA – not available  Ig – immunoglobulin

referring hospital. (Table 59 ). All but one initial lymphocyte counts were low, the number on first presentation being less than $1.5 \times 10^9/l$ in five of eight infants. In all the infants presenting with more than $1.5 \times 10^9$ lymphocytes/litre, the lymphocyte count progressively fell. Moreover, T and B lymphocyte numbers were not always low from first testing and also showed this progressive decline although only two of the results in Table 59 were obtained at less than 10 weeks of age. Long term gnotobiotic infants with SCID have been described (Teller 1973; Williamson 1977). All three maintained their initial low lymphocyte counts throughout the first year of life (table 58a). These data

Table 58a

| patient | ER | WR | DV | Normal Range |
|---|---|---|---|---|
| Lymphocyte count $\times 10^9/l$ at | | | | |
| birth | 1.0 | 1.4 | 1.5 | 3.0–10.0 |
| 4 weeks | 2.0 | 2.6 | 3.8 | 2.7–8.5 |
| 13 weeks | 1.8 | 2.0 | 2.0 | |
| 22 weeks | 1.3 | 2.2 | 2.0 | |
| 36 weeks | 1.0 | 1.7 | 2.0 | 2.8–7.7 |
| 52 weeks | 1.8 | 2.0 | 2.3 | |
| probable inheritance | autosomal recessive | autosomal recessive | sex-linked | |

with the data from the eight infants do not suggest any particular pattern of involution of lymphocyte counts with different patterns of inheritance, although involution of non-maternal derived immunity occurs in the immune deficiency associated with adenosine deaminase (ADA) deficiency (Polmar 1977). Normal or only slightly reduced lymphocyte or lymphocyte sub-populations do not exclude the diagnosis of SCID, and lymphocyte function studies are more reliable.

Lymphocyte function studies

T lymphocyte function was severely depressed in all patients as assessed by the uptake of tritiated thymidine in response to phytohaemagglutinin (PHA), allogeneic cells and candida immunogen (table 60 ). In addition no skin tests for delayed hypersensitivity were positive, and in the two children who had biopsy of a lymph node, the node was vestigial.

Bortin and Rimm (1977) reported that none of 47 infants had

Table 59

SEQUENTIAL STUDIES OF LYMPHOCYTE POPULATIONS IN SCID

| Patient | Age (weeks) | Lymphocytes $\times 10^9/l$ | T lymphocytes $\times 10^9/l$ | B lymphocytes $\times 10^9/l$ |
|---|---|---|---|---|
| MP | 13 | 1.44 | | |
| | 16 | 1.75 | | |
| | 20 | 2.5 | | |
| | 22 | 1.6 | | |
| JWh | 13 | 4.0 | | |
| | 22 | 1.2 | | |
| | 25 | 1.0 | | |
| TD | 18 | 0.78 | | |
| | 20 | 2.20 | | |
| | 25 | 4.76 | | |
| | 26 | 0.65 | | |
| | 28 | 1.26 | | |
| GS | 9 | 1.18 | | |
| | 12 | 0.28 | | |
| | 14 | 0.42 | | |
| | 16 | 0.35 | | |
| DF | 32 | 2.2 | 0.04 | |
| | 37 | 0.53 | < 0.01 | |
| LS | 7 | 0.78 | 0.08 | 0.15 |
| | 15 | 0.24 | 0.02 | |
| AJ | 45 | 1.0 | 0.19 | 0.03 |
| | 49 | 0.94 | 0.15 | NA |
| | 50 | 0.16 | 0.03 | 0.01 |
| | 51 | 0.33 | 0.1 | 0.04 |
| | 56 | 1.20 | 0.23 | 0.05 |
| SI | 13 | 2.02 | NA | NA |
| | 22 | 1.82 | 0.04 | 1.1 |
| | 37 | 0.50 | 0.03 | 0.1 |

Lymphocytes NR 6–15 weeks 2.7–8.5 $\times 10^9/l$
15–40 weeks 2.8–7.7 $\times 10^9/l$

T lymphocytes NR 5–26 weeks 0.08–0.7 $\times 10^9/l$
26–52 weeks 0.18–1.0 $\times 10^9/l$

B lymphocytes NR 0.1–1.0 $\times 10^9/l$

NR normal range

NA not available

Table 60

## HETEROGENEITY OF SCID

Cell mediated immunity at presentation to WCH

| | | Increments (cpm) in autologous plasma to | | | |
|---|---|---|---|---|---|
| Patient | age (weeks) | PHA | allogeneic cells | candida immunogen | skin tests |
| TD | 16 | 209 | 0 | 0 | DNCB neg. |
| LS | 16 | 2400 | 1600 | 110 | No DHS |
| GS | 18 | 30 | 0 | 0 | DNCB neg.<br>No DHS[1]<br>No lymphocytes in lymph node biopsy |
| MP | 22 | 17 | 0 | 0 | |
| JWh | 25 | no response | ND | ND | |
| HN | 25 | 0 | 250 | 450 | |
| DF | 37 | 240 | 2 | 20 | |
| SI | 37 | severely reduced* | severely reduced* | severely reduced* | Heaf neg.[2]<br>No DHS[1] |
| AJ | 50 | 100 | 220 | 480 | |
| PL | 70 | 0 | 60 | ND | DNCB neg.<br>No DHS<br>No lymphocytes in node biopsy |
| Normal range | | 7000+ | 7000+ | 2000+ | |

[1] infected with candida

[2] given BCG at birth

* assessed elsewhere

DHS - delayed hypersensitivity

a positive delayed hypersensitivity skin test on 138 test occasions. In-vitro proliferative responses expressed as the mean uptake of thymidine compared to control were as follows

| Stimulating agent | Mean uptake (% of control) | |
|---|---|---|
| | Registry patients | WCH patients |
| PHA | 3% | 5.5% |
| allogeneic cells | 6% | 6.7% |
| candida immunogen | 6% | 7.5% |

The Westminster results very much parallel the larger reported series, variation in response to different stimuli being attributed to patients having different residual activity in their T lymphocytes or to the allogeneic stimulating cells supplying unknown essential metabolites to the test lymphocytes. Either way, the results in both series show severe impairment.

Except in comparison with the International Registry series above, I have chosen to report the increments shown by the test lymphocytes over the background. If the background count is unusually high a stimulation index expressing the ratio of response obtained from the test lymphocytes divided by the background response will be disproportionately low. In addition the tests were performed in both autologous and AB plasma. AB plasma avoids the effects of free inhibitors in the patient's plasma but would not affect lymphocytes with inhibitors already surface bound. There was no difference between the results obtained in autologous or AB plasma so the results quoted are those obtained in autologous plasma.

Heterogeneity was more evident in B lymphocyte function. The number of lymphocytes bearing surface immunoglobulin was normal in four of eight infants (table 61). Immunoglobulin (Ig) G levels were usually very low and reflected maternally derived immunoglobulin or replacement therapy. IgA levels were usually minimal and the only immunoglobulin present in any quantity was the IgM present in four of eleven patients. (Table 61). However only one had any competent IgM in that he has an anti-B isohaemagglutinin titre of 1/4 at 6 months of age (table 62). This would usually be detectable at 8 weeks of age. Despite salmonella dublin in the stool for some weeks neither he nor

Table 61

## HETEROGENEITY OF SCID

Humoral immunity at presentation to WCH

| Patient | Age weeks | No. of B lymphocytes x $10^9$/l | Immunoglobulins (gms/l) | | | |
|---|---|---|---|---|---|---|
| | | | G | A | M | E units/ml |
| TD | 16 | ND | 1.4 | <.01 | .08 | ND |
| LS | 16 | 0.12 | 1.4* | <.01 | <.05 | 34 |
| GS | 18 | 0.35 | 0.25 | <.01 | .09 | ND |
| MP | 22 | ND | 0.55 | .03 | .05 | 350 |
| JWh | 25 | 0.12 | 0.25 | .06 | .4 | ND |
| HN | 25 | 0.02 | 1.2* | <.01 | .1 | <10 |
| DF | 37 | 0.01 | 0.5 | <.01 | <.1 | 18 |
| SI | 37 | 0.41 | 1.7* | <.02 | .5 | 4 |
| AJ | 50 | 0.01 | 0.15 | <.01 | <.05 | 47 |
| PL | 70 | 0.8 | 1.4* | .27 | .37 | NA |
| DV** | 26 | NA | 1.2 | <.01 | .22 | NA |
| Normal range | 16-52 | 0.1-1.0 | 2-10 | 0.1-0.9 | 0.2-2.0 | <35 |

* on replacement therapy ** gnotobiotic

ND not done NA not available

Table 62

COMPETENT ANTIBODY PRODUCTION

| Patient | Age (weeks) | Blood group | Antibodies | Complement |
|---|---|---|---|---|
| TD | 16 | O | No anti-A or Anti-B | 2 |
| LS | 16 | O | No anti-A or Anti-B<br>No antitetanus antibodies[1] | 2<br>C1q 35% of normal |
| GS | 18 | O | No anti-A or anti-B | No germinal centres in lymph node |
| MP | 22 | A | No anti-B<br>No salivary IgA | 2 |
| JWh | 25 | A | Anti-B 1/4<br>Widal negative[3] | ND |
| HN | 25 | A | No anti-B<br>No salivary IgA | 2 |
| DF | 37 | A | No anti-B<br>Widal negative[3] | 2 |
| SI | 37 | B | No anti-A | 2<br>C1q 54% of normal |
| AJ | 50 | O | No anti-A or B<br>Schick reactive[1] | 2<br>No germinal centres in lymph node |
| PL | 70 | O | No anti-A or B | NA |
| DV** | 26 | A | No anti-B | 2<br>C1q 30% of normal |

NA not available ** gnotobiotic

[1] received diphtheria/tetanus immunisation previously

[2] $C_3$ $C_4$ were normal

[3] infected with salmonella spp.

ND not done

the infant with salmonella typhi septicaemia had salmonella antibodies. Two infants who received diphtheria and tetanus toxoids could neither neutralise intradermal diphtheria toxin nor produce anti-tetanus antibodies unlike many normal infants aged 4 weeks (Smith and Eitzman 1964). In the Registry report (Bortin and Rimm 1977) 96% of infants had sub-normal IgG levels, 91% subnormal IgA levels and 80% subnormal IgM levels. A few had isohaemagglutinins but the titres were less than 1/4. Twenty-three of 29 infants produced no antibody in response to any immunisation and those who did produce a four-fold antibody titre rise did this to only one of the administered antigens.

Complement $C_3$ and $C_4$ levels were normal whenever tested but C1q levels were subnormal in the three infants assessed. No salivary immunoglobulin was found in the two infants assessed and both had free secretory piece present in their saliva. These findings have been reported in many cases of SCID and each has become normal after a successful transplant, as have chemotactic defects (Pahwa et al 1978).

Sequential studies of immunoglobulin levels on four patients showed the expected decline in IgG levels (table 63 ). IgA levels varied in P.L. who received maternal plasma as did IgM levels in both P.L. and S.I. Despite P.L. having almost normal IgM levels for seven months, not once was he shown to have competent antibody.

Laboratory diagnosis of SCID

This heterogeneity does not give much diagnostic confusion in SCID but more difficulty may be experienced with milder cases. If in doubt then repeat of the most important investigations after two weeks will almost certainly resolve the problem. The history, family history and examination accompanied by the following investigations should make the diagnosis clear (table 64 ).

Specific radiological abnormalities in SCID

Classically the thymus is absent in SCID. However this absence is not always real as the thymic shadow frequently appears about three months after a successful marrow transplant. In these cases, the thymus must have remained vestigial and

Table 63

SEQUENTIAL IMMUNOGLOBULIN LEVELS

| Patient | age in weeks | Immunoglobulins gms/l | | |
|---|---|---|---|---|
| | | G | A | M |
| LS | 3 | 4.2 | < 0.1 | < 0.1 |
| | 5 | 4 | < 0.1 | < 0.1 |
| | 15 | 1.4* | < 0.1 | < 0.1 |
| AJ | 49 | 0.1 | < 0.1 | < 0.1 |
| | 50 | 0.15 | < 0.1 | < 0.1 |
| | 56 | 2.6* | < 0.1 | < 0.1 |
| SI | 22 | 2 | 0.2 | 0.4 |
| | 24 | 0.2 | 0.05 | 0.41 |
| | 28 | 1.5* | 0.05 | 1.4 |
| | 37 | 1.7 | 0.05 | 0.5 |
| PL | 14 | 0.86 | 0.05 | 0.18 |
| | 18 | 0.7* | 0.05 | < 0.1 |
| | 22 | 1.0 | 0.2 | < 0.1 |
| | 26 | 0.8 | 0.23 | 0.35 |
| | 30 | 0.5 | 0.1 | 0.26 |
| | 40 | 0.9 | 0.1 | 0.41 |
| | 48 | 0.86 | 0.05 | 0.42 |
| | 57 | 1.4 | 0.27 | 0.37 |

* on replacement Ig then and thereafter

Normal Values

| age in weeks | Immunoglobulin G gms/l | IgA gms/l | IgM gms/l |
|---|---|---|---|
| 2-4 | 3.5-12 | < 0.1-0.2 | 0.1-0.3 |
| 4-6 | 3.5-12 | < 0.1-0.2 | 0.1-0.5 |
| 6-12 | 2-7.5 | 0.1-0.5 | 0.1-0.7 |
| 12-26 | 2-8 | 0.1-0.7 | 0.2-1.4 |
| 26-39 | 2.5-8.5 | 0.2-0.85 | 0.4-1.8 |
| 39-52 | 3-10 | 0.3-0.9 | 0.5-2.0 |
| 52-104 | 3-13 | 0.4-1.3 | 0.5-2.0 |

Table 64

## INVESTIGATION OF POSSIBLE SCID

Basic

Lymphocyte numbers

* T lymphocyte numbers

B lymphocyte numbers

* Immunoglobulins G, A and M

Isohaemagglutinin titres

* DHS to mumps, candida, trichophyton, SK-SD

* Transformation to PHA, allogeneic cells and candida immunogen

Chest X-ray (PA and lateral)

* normal results in these tests would be against the diagnosis.

Additional

Salivary IgA (present after age 18 weeks)

Complement C1q

Response to immunisation with diphtheria and tetanus toxoid

Adenosine deaminase, nucleoside phosphorylase, co-carboxylase and transcobalamin II levels

Other

Antibody generation by autologous B cells

Chemotactic studies

Lymph node biopsy

Thymic biopsy (Borzy et al 1979)

DNBC, KLH skin testing

Skin grafting from third party

Platelet function studies

failed to populate until it was presented with competent lymphocytes. Any infant subjected to major stress may lose the thymic shadow on chest x-ray in 72 hours - thus the absence of a thymus on chest x-ray of a sick infant does not always indicate SCID. When the stress is past, the thymus shadow will re-appear.

Radiological changes associated with ADA deficient SCID have been described by Meuwissen et al (1975). There is splaying and cupping of the costochondral junctions and sclerotic lines in the iliac apophyses. Wide vertebral disc spaces with increased height of the vertebrae and short stubby ilia with widening of the metaphyses were also described by Alexander and Dunbar (1967). The absence of a zone of proliferating cells at the metaphyseal/epiphyseal border seems to be characteristic of SCID with adenosine deaminase deficiency and is unlike the abnormalities in the chondrodysplasia with immunodeficiency syndromes (Lederbaum et al 1976).

Apart from absence of thymic shadow, none of these cases has demonstrated any of the other radiological abnormalities, even those associated with adenosine deaminase deficiency.

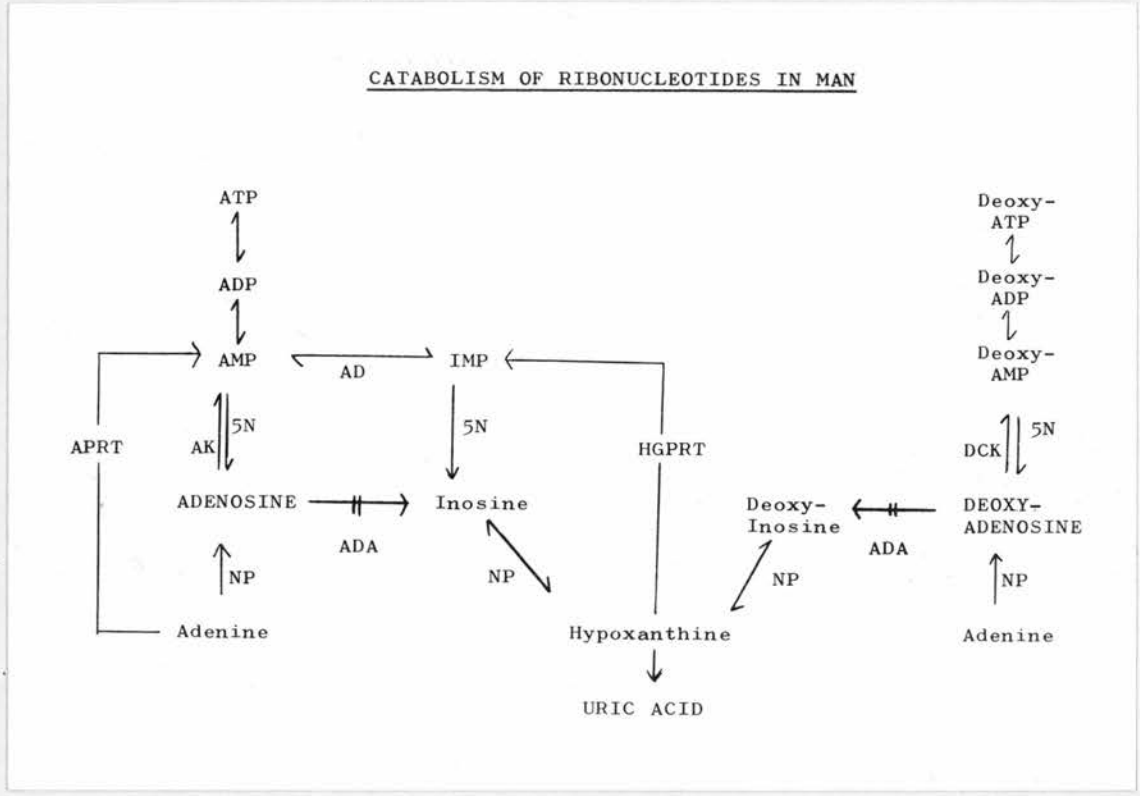
Metabolic disorders associated with immune deficiency

Five metabolic disorders have been associated with primary immunodeficiency. These are deficiency of the enzymes adenosine deaminase (ADA), nucleotide phosphorylase, 5-nucleotidase, biotin cocarboxylase and absence of transcobalamin II. ADA deficiency affects 16% of infants with SCID (Bortin and Rimm 1977) and was the first enzyme deficiency to be associated with immunodeficiency. (Giblett et al 1972).

ADA catalyses the conversion of adenosine to inosine and hence to elimination via hypoxanthine. It also catalyses the conversion of the corresponding deoxynucleosides to the same final pathway. (Fig. 11 ). Inactive forms of the enzyme may exist (Hirschhorn et al 1976) and a labile enzyme inhibitor has been suggested to explain why lysed erythrocytes from some patients and obligate heterozygotes have normal activity after prolonged storage (Trotta et al 1976). Rare cases of deficiency of ADA without immune deficiency have been reported (Jenkins et al 1976) and erythrocyte ADA deficiency may be dissociated from other tissue deficiency.

Fig. 11

# ADENOSINE DEAMINASE IN PURINE METABOLISM



The histopathology of the thymus differs in ADA deficient SCID. In conventional SCID a primitive embryonal epithelium without Hassall's corpuscles is usually found but in ADA deficiency the central epithelium is differentiated, Hassall's corpuscles are present and the blood vessels are relatively large, suggesting that involution has occurred. (Polmar 1977).

In patient L.S., the only one with ADA deficiency, family studies confirmed her parents as heterozygotes in that they both had reduced enzyme activity. Lymphocyte-ADA levels were not studied pre-graft on account of the infant's severe lymphopenia.

ADENOSINE DEAMINASE ACTIVITY IN FAMILY OF PATIENT L.S.

| | Red cells | Fibroblasts |
|---|---|---|
| Patient L.S. | 0.59 | 0.11 |
| Father | 33.4 | 0.65 |
| Mother | 36.8 | 0.93 |
| Adult controls n = 9 | 36-106 | 1.3-2.3 |

results quoted in $\mu$mols/mg protein/hour

ADA replacement therapy in L.S.

During the search for a donor, three separate exchange transfusions using irradiated nitrogen frozen erythrocytes supplied on each occasion an ADA activity of 65$\mu$mols/mg haemoglobin/hour. The technique used two simultaneous butterfly needles (23G) over half an hour to remove sufficient blood to give about 25 mls/kg of ADA containing red cells, with a post-transfusion haemoglobin of 14 gms/dl. This was a messy inconvenient procedure using peripheral veins but the central venous feeding line was not be be compromised by the passage of blood.

EFFECT OF ADA CONTAINING EXCHANGE TRANSFUSIONS

| age (weeks) | volume given mls/kg | other therapy | Increments (cpm) in response to | |
|---|---|---|---|---|
| | | | PHA | Allogeneic cells |
| 10 | 0 | | 2400 | 9300 |
| 15 | 0 | | | 1500 |
| 17 | 32 | | | |
| 19 | 26 | daily thymosin for 14 days | 70 | 140 |
| 21 | 24 | | 2900 | 60 |

The number of lymphocytes and T lymphocytes remained unchanged. The level of ADA in the infant's erythrocytes (1.6% of normal) was much less than that of the children treated successfully by Polmar et al (1976) and is similar to the levels of those who responded poorly (Schmalsteig et al 1978). In-vitro addition of ADA to the infant's lymphocytes produced no response. Within two weeks of the first red cell transfusion, the patient of Polmar et al (1976) had a normal lymphocyte count and normal in-vitro responses to PHA, Concanavalin-A, pokeweed mitogen and allogeneic cells. Subsequent transfusions produced less effect but normal plasma followed by red cell transfusion restored the effect. Rubenstein et al (1979) reported an 18 month old child who responded to frozen red cells when thymosin was also given, whereas his 6 week old affected sibling responded to only red cells. A sixfold increase in PHA increments was seen at two weeks without change in cell counts, so it is possible that L.S. was responding to red cell infusions. In the meantime an unrelated donor who was HLA-A,B,C compatible and MLC unreactive was found. ADA replacement therapy was discontinued and bone marrow grafting performed.

Purine studies following transplantation

Mills et al (1976) stated that adenine was the characteristic urinary metabolite in the ADA deficient SCID and that urine examination would screen satisfactorily for ADA deficiency. Using urinary isotachophoresis, Dr. Anne Simmonds of Guy's Hospital demonstrated that adenine was not initially present in the urine of that Texas patient but was a degradation product of adenosine and deoxy-adenosine. (Simmonds et al 1978). In-vitro studies (Polmar et al 1977; Simmonds et al 1978) demonstrated that both adenosine and deoxyadenosine are toxic to dividing cells. The concentration of adenosine require to inhibit lymphocyte proliferation was 100 times greater than that of deoxyadenosine (1$\mu$mol/l) and this level of deoxyadenosine was close to that finally demonstrated in the patient reported by Mills et al. It therefore seemed most likely that deoxyadenosine rather than adenosine was the toxic factor present and this was confirmed in my patient L.S. (table 65 ) before treatment. Successful transplantation was associated with loss of deoxy-

Table 65

URINARY PURINES IN L.S.

| | pre therapy | days post transplant | | | | | controls age 4 |
|---|---|---|---|---|---|---|---|
| | | +51 | +65 | +83 | +102 | +193 | |
| total urinary purine bases | 1.71 | 1.06 | 1.32 | .87 | 1.21 | .89 | 0.04–0.94 |
| urinary adenine | UD | UD | UD | UD | UD | UD | UD |
| urinary deoxyadenosine | .19 | UD | UD | .006 | .001 | UD | UD |

results quoted as mmols/mmol creatinine

UD – undetectable

Table 66

ERYTHROCYTE NUCLEOTIDE LEVELS IN L.S.

| | ATP | ADP | AMP | dATP | dADP | dAMP |
|---|---|---|---|---|---|---|
| pre treatment | 760 | 135 | 30 | 750 | 116 | 15 |
| days post transplant +51 | 832 | NE | 30 | not detected | | |
| days post transplant +65 | 1020 | 214 | 52 | not detected | | |
| days post transplant +84 | 1133 | 133 | 16 | not detected | | |
| days post transplant +100 | 1340 | 137 | 15 | not detected | | |
| days post transplant +200 | 1260 | 131 | 10 | not detected | | |
| days post transplant +234 | 1190 | 210 | 6 | not detected | | |
| control | 1278 ±127 | 114 ±24 | 10 ±3 | not detected | | |

μmols/ml packed red cells

NE – not evaluable

adenosine from the urine.

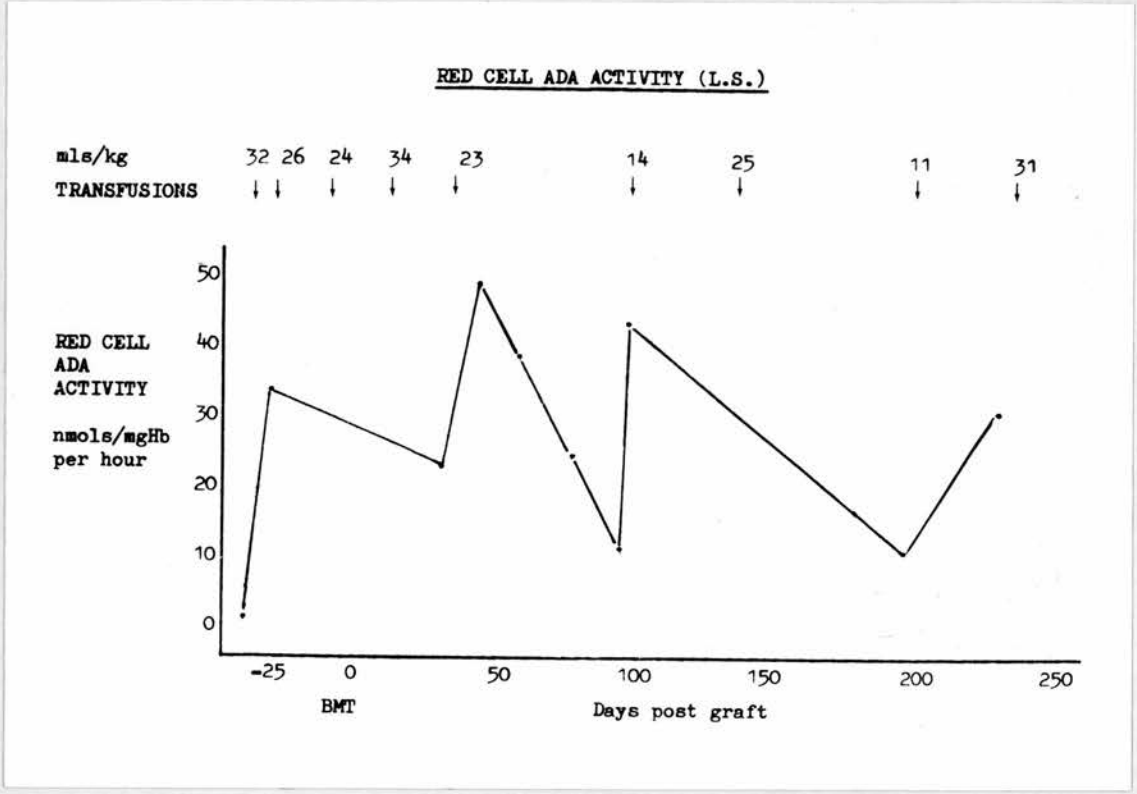
Erythrocyte nucleotides were also studied (table 66). Large amounts of different deoxy-nucleotides were present in the erythrocytes before therapy and these could not be detected after successful marrow transplantation. The presence of the deoxy compounds in a similar ratio to that of the oxy compounds was a new observation and this infant was the first in whom deoxy AMP had been detected.

Erythrocyte ADA activity was raised after the transplant and it is possible that this was responsible for elimination of the toxic nucleosides. Erythrocyte ADA activity after grafting could only have arisen from transfusions or the transplant, but there is only one reported case of red cell engraftment in SCID (Rubenstein et al 1971). Erythrocyte ADA levels (fig. 12) show no tendency to a steady level as should occur in a stable chimera and in particular show a steady decline between days 50 and 100 after grafting during which no transfusions were given. It seems most unlikely that a red cell graft occurred and in particular erythrocyte genotyping and iso-enzyme studies on days 100 and 200 showed no factors which could not have been parentally derived. There was therefore no specific evidence of a red cell graft, and all red cell ADA activity must have been derived from transfusions.

Lymphocyte ADA activity was studied only after transplantation (table 67). It is likely that this lymphocyte activity was the result of engraftment although no C or Q banding was performed and tissue heterogeneity of ADA activity has been described. Lymphocytes are implanted in successful transplants and ADA deficient infants given red cell exchange transfusions do not alter their lymphocyte ADA levels (Polmar 1979). The decline in lymphocyte ADA activity to 10% of the minimum of the control values was not associated with impairment of T lymphocyte responses but B lymphocyte function progressively fell from day 120. Ultimately T lymphocyte function also declined and the graft failed.

## ERYTHROCYTE ADA LEVELS

Fig. 12




## LYMPHOCYTE ADA ACTIVITY

Table 67

| Pre-treatment | not measured |
|---|---|
| Days post graft | |
| + 100 days | 31% |
| + 234 days | 10% |

expressed at % of lower limit of control

## MARROW TRANSPLANTATION FOR SEVERE COMBINED IMMUNE DEFICIENCY

As soon as the diagnosis of SCID has been made, arrangements for tissue typing of the infant and immediate family should proceed as quickly as possible. In the United Kingdom there is an even chance of an older living sibling but only a 25% chance that this sibling will be fully compatible, i.e. one infant in eight should have a compatible sibling. If the parents were previously related, appear to share one haplotype or are apparently homozygous at any HLA locus then uncles and aunts should also be tested. Haplotypic identity outwith the immediate family is only such as can be defined by in-vitro testing and cannot imply full MHC identity. Several successful HLA-A,B incompatible but D compatible transplants have been reported (O'Reilly et al 1978), and if no HLA compatible relative is found, it is still worth performing mixed lymphocyte cultures between the infant and the immediate family in addition to seeking an unrelated but compatible volunteer and before indulging in immunological modification of the parents.

### Transplant details

| | |
|---|---|
| One bone marrow graft | six patients |
| Two bone marrow grafts | one patient |
| Fetal liver graft | one patient |
| Graft from blood transfusion | one patient |
| Died pre-graft | one patient |
| Still in isolation awaiting transplant, now age nine (Houston, Texas) | one patient |

Of my 11 infants, four had no compatible family or unrelated volunteer donor and therefore did not receive a bone marrow transplant. The infant accidentally grafted by a washed red cell transfusion at the referring hospital died of acute GVHD. This has been reported on several occasions previously and is a well recognised hazard of severe cellular immunoincompetence. Of the seven infants who received bone marrow transplants (table 68) two (18%) had a compatible sibling and two had a compatible father. A compatible unrelated volunteer was found for one infant and two had an aunt with one locus mismatched. All donor/recipient pairs seemed compatible in MLC as indicated by an increment of less than 500 cpm over the unstimulated background, despite two of the seven infants having a known HLA-A, or B mismatch with their donor.

The technique of marrow harvest has been described by

Table 68

TRANSPLANTS FOR SCID. DONORS AND RECIPIENTS

| Patient | Sex | Age (weeks) | Donor | Age | Donor vs patient (m) | | |
|---|---|---|---|---|---|---|---|
| | | | | | c.p.m. | increment | stimulation index |
| T.D. | F | 30 | Brother | 4 | 186/332 | 0 | 0.56 |
| L.S.(1) | F | 21 | Unrelated | 30 | 400/372 | 28 | 1.08 |
| L.S.(2) | F | 58 | Unrelated | 30 | 73/421 | 0 | 0.17 |
| G.S. | M | 19 | Step-sister | 7 | 69/68 | 1 | 1.01 |
| M.P. | M | 26 | Father | 24 | 95/52 | 43 | 1.83 |
| H.N. | F | 29 | Maternal Aunt | 14 | 545/156 | 389 | 3.49 |
| D.F. | F | 37 | Paternal Aunt | 24 | 219/135 | 74 | 1.62 |
| S.I. | M | 36 | Father | 35 | 117/1012 | 0 | 0.11 |
| | | | | ACCEPTABLE | <500 | | 2 |

(M) mitomycin blocked

Barrett and Humble (1975). The packed marrow was usually given with frusemide through a platelet giving set, the infusion being completed within 6 hours of marrow harvest. Trypan blue exclusion showed greater than 95% viability of the infused cells. The volume and cell content of each transplant is shown below

| patient | total volume infused (mls) | nucleated cells/kg recipient body weight |
|---|---|---|
| TD | 13 | $0.68 \times 10^8$ |
| LS (1) | 60 | $3.2 \times 10^8$ |
| LS (2) | 200 | $13 \times 10^8$ |
| GS | 40 | $5.5 \times 10^8$ |
| MP | 40 | $3.01 \times 10^8$ |
| HN | 100 | $10 \times 10^8$ |
| DF | 20 | $0.68 \times 10^8$ |
| SI | 250 (unpacked) | $14.5 \times 10^8$ |
| mean | 90 | $6.31 \times 10^8$ |

The mean graft of $6.3 \times 10^8$ nucleated cells/kg recipient body weight is higher than necessary. No immunological disadvantage is known to result from this in SCID but the amounts required to graft an infant with SCID are much smaller than the $3 \times 10^8$ nucleated cells/kg recommended in aplastic anaemia. (Storb et al 1978). No preconditioning was used although this is occasionally necessary to overcome "non-lymphoid cellular resistance" (O'Reilly et al 1977).

Five transplants showed evidence of engraftment including two infants who died at 7 and 37 days after grafting. Three infants died at days 13, 18 and 23 with no evidence of graft-take. Prophylaxis against GVHD was given on five occasions, either using methotrexate in the Seattle schedule (Storb et al 1972) or cyclosporin-A (Powles et al 1980). To date other special techniques to avoid GVHD in grafting for SCID have either been unsuccessful or have not resulted in full immune reconstitution. Definite GVHD occurred in two of four infants who survived in excess of 20 days and was fatal in the one in whom the most vigorous reaction was predicted by the in-vitro assessment (patient HN table 68 ).

Recovery after grafting

This was seen both clinically and on laboratory investigation. The first sign is an improvement in the diarrhoea, usually at about 10 days and the infant becomes less miserable. Subsequently weight is gained, infected sites heal, areas with candidiasis show a local reaction and slowly lymph nodes become palpable and tonsils visible. Delayed hypersensitivity challenge sites may become positive. A thymic shadow may appear on chest x-ray after three months. The peripheral blood may show a reticulocytosis during the second week, followed by a slowly rising lymphocyte count though it usually remains subnormal.

Early deaths

In this series of eight marrow transplants in seven infants, five died within 37 days of a marrow graft (table 69 ). Three showed no evidence of graft-take. S.I. died of BCG disease derived from a neonatal immunisation despite 10 days of treatment with streptomycin, rifampicin and isoniazid. He received cyclosporin-A and methotrexate as prophylaxis against GVHD. D.F. died of overwhelming pseudomonas aeruginosa infection at day 13. LS (2) probably died of pneumocystis carinii pneumonia. An earlier severe neutropenia was attributed to co-trimoxazole which she had received as prophylaxis against pneumocystis. She also received cyclosporin-A and methotrexate as prophylaxis against GVHD. The events following her first graft are discussed in this chapter.

Two infants who died had evidence of engraftment (table 69 ). G.S. developed an acute duodenal ulcer with a major concealed haemorrhage. H.N. had a skin rash, biopsy of which showed mild GVHD. She developed two-to-one heart block on day +32 which rapidly progressed and despite transvenous pacing she died 5 days later of myocardial failure. No viruses were found and antibody studies were unhelpful. Lymphocytes were found inside the sarcolemmal sheaths as well as in the other tissues mentioned. She had normal numbers of T and B lymphocytes by day 27 with normal immunoglobulin levels when she died (Table 70 ).

Table 69

SCID PATIENTS DYING - EVIDENCE OF RECONSTITUTION

| Patient | Day of death after marrow graft | Cause of death | Evidence for reconstitution |
|---|---|---|---|
| DF | +13 | Pseudomonas septicaemia and pneumonia | None (no autopsy) |
| SI | +18 | Tuberculous bronchopneumonia and systemic BCG disease | Normal haemopoietic marrow, no peripheral or thymic lymphocytes |
| $LS_2$ | +23 | Pneumonitis ?pneumocystis | None (no autopsy) |
| GS | +7 | Curling's ulcer, massive haemorrhage | Lymphocytes in lymph nodes |
| HN | +37 | Myocarditis, glossitis, thyroiditis, pneumonitis, GVHD | Lymphocyte count normal<br>Lymphocyte invasion of tissues<br>Normal immunoglobulin levels |

Table 70

PATIENT H.N. - PROGRESS POST GRAFT

| | Lymphocytes $\times 10^9/l$ | T lymphocytes $\times 10^9/l$ | B lymphocytes $\times 10^9/l$ |
|---|---|---|---|
| Pregraft | 0.288 | 0.014 | 0.015 |
| Days post graft | | | |
| +14 | | | |
| +20 | | | |
| +30 | 0.777 | 0.194 | 0.225 |
| +36 | 1.655 | 0.28 | 0.530 |
| NR | 2.8–13.6 | 0.180–1.0 | 0.1–1.0 |

NR - normal range

Immunoglobulins (gms/l)

| | G | A | M |
|---|---|---|---|
| Pregraft | 1.2 | ND | 0.1 |
| Days post graft | | | |
| +14 | 1.9 | 0.1 | 0.1 |
| +20 | 2.0 | 0.2 | 0.1 |
| +30 | 2.6 | 0.3 | 0.2 |
| +36 | 4.2 | 0.7 | 0.4 |
| NR | 2.5–8.5 | 0.2–0.8 | 0.4–1.8 |

NR - normal range ND - not detected

Prolonged engraftment

Three infants reconstituted completely, two of whom remain well seven and eight years later. The other infant $LS_{(1)}$ showed reconstitution but the graft did not persist beyond day 230.

1) TD received a transplant from her compatible brother aged 4 years. (Fig. 13 and 14). She has been fully reported (Yamamura et al 1972). Mastoiditis the day after grafting was followed by rapid clinical improvement, cessation of diarrhoea and weight gain within the first month. Immune reconstitution was rapid although in-vitro T lymphocyte function remained impaired for many months. Details of this reconstitution are shown in tables 71 and 72. Return of salivary immunoglobulin A was delayed until 6 months after the graft, coinciding with improvement in her chronic cough.

She has had three episodes of pneumonia in different areas in the past 6 years and had a fairly severe attack of chickenpox. Otherwise her progress has been unremarkable and she attends a normal school. Her lymphocytes remain XY on karyotype assay, but her polymorphs retain nuclear clubs.

2) MP received marrow from his compatible father. The two photographs (fig. 15 and 16 ) show MP before his transplant and about 12 months later with his donor. Table 73 shows his lymphocyte reconstitution and normal immunoglobulin levels by 5 weeks. He had a severe hepatitis due to GVHD between days 50 and 500. This was reflected in some depression of his immunoglobulins at that time. Qualitative antibody was present when tested at 811 days. He had pneumonia at 880 days from which he made a full recovery. Figure 17 shows his response to PHA, allogeneic cells and candida immunogen expressed as the increment in cpm over background. The depressed results at 660 days bore no relation to his excellent health. Mixed lymphocyte cultures between the child and his father were completely unreactive at 22 and 83 months, yet showed good responses against a third party.

PATIENT T.D.

Fig. 13

PRE GRAFT

POST GRAFT WITH HER BROTHER (THE DONOR)

Fig. 14

Table 71

T.D. - IMMUNE RECONSTITUTION

| | lymphocytes x $10^9$/l | Immuno-globulins (gms/l) | | | Antibodies |
|---|---|---|---|---|---|
| | | G | A | M | |
| Pre graft | 0.6 | 1.4 | .01 | .08 | no isohaemagglutinins |
| days post graft | | | | | |
| +7 | 0.88 | | | | |
| 21 | 2.10 | 7.0 | 0.08 | 0.2 | |
| 60 | 2.31 | 8.2 | 0.04 | 0.12 | |
| 120 | 2.43 | 3.9 | 0.04 | 1.9 | |
| 150 | 1.27 | 4.5 | 0.03 | 2.5 | |
| 180 | 0.72 | 6.8 | 0.05 | 2.4 | |
| 210 | 1.40 | 5.5 | 0.7 | 0.56 | E coli antibodies present |
| +125 weeks | 4.00 | 5.4 | 1.2 | 1.5 | Anti-A 1/64 anti-B 1/32 tetanus and polio antibodies present following immunisation |

Table 72

T.D. CELL MEDIATED IMMUNE RECONSTITUTION

| | PHA | MLR |
|---|---|---|
| pre graft | 6% of control | 2% of control |
| days post graft | | |
| +30 | 8% of control | |
| +60 | 63% of control | 60% of control |
| +120 | | 42% of control |
| +140 | | 50% of control |
| + 334 weeks | 170% of control | 700% of control |

PATIENT M.P. Fig. 15

PREGRAFT

ONE YEAR POST GRAFT with donor Fig. 16

Table 73

IMMUNE RECONSTITUTION M.P.

| | Lymphocytes $\times 10^9/l$ | Immunoglobulins G | A gms/l | M |
|---|---|---|---|---|
| pre graft | 1.22 | 0.55 | 0.03 | <0.05 |
| days post graft | | | | |
| + 9 | 1.06 | | | |
| 14 | 0.96 | | | |
| 21 | 1.79 | | | |
| 31 | 2.25 | 5.1 | 1.01 | 1.85 |
| 44 | | 9.2 | 1.45 | 1.15 |
| 55 | 3.72 | | | |
| 183 | | 2.9 | 0.11 | 1.08 |
| 661 | 1.68 | 5.9 | 0.3 | 0.7 |
| 811 | 3.74 | 6 | 0.7 | 0.9 |
| + 137 weeks | | 5.6 | 0.4 | 0.7 |
| + 5 years | | 6.8 | 0.3 | 0.7 |

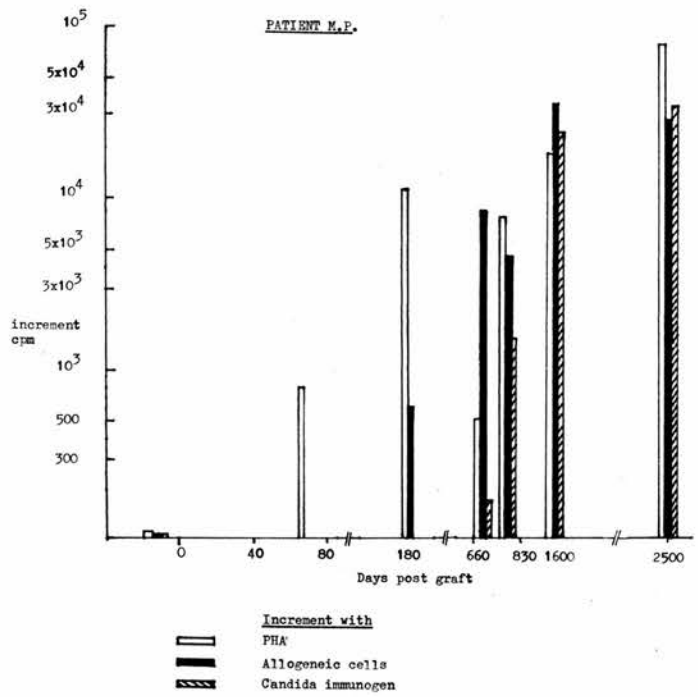
Normal range
from day 960
IgG 5-14 gms/l
IgA 0.5-1.8 gms/l
IgM 0.5-2 gms/l
Lymphocytes 2.5-6.2 $\times 10^9/l$

Isohaemagglutinins
tetanus and polio antibodies
present at day 811

PATIENT M.P. Fig. 17

RECOVERY OF T LYMPHOCYTE FUNCTION

Increments to PHA, allogeneic cells and candida immunogen



3) $LS_{(1)}$ received her transplant from an unrelated female volunteer who was HLA-A,B,C compatible and MLC unreactive on two separate occasions. Cyclosporin-A and methotrexate were given as prophylaxis against GVHD. Her reconstitution (tables 74 and 75 , figure 18 ) may have been slowed by the GVHD prophylaxis regimen. No $G_m$ or $K_m$ allotypic immunoglobulin marker which was present in the donor and not present in either parent was identified. Chromosome banding was not done, so absolute proof of engraftment is lacking.

Table 74 shows lymphocyte repopulation and immunoglobulin levels after her transplant; table 75 the qualitative antibody and clinical delayed hypersensitivity tests; and figure 18 the increments following stimulation of her T lymphocytes by PHA, pooled allogeneic cells and candida immunogen.

Reconstitution of lymphocyte numbers was never very impressive. T lymphocyte numbers became normal at 70 days but B lymphocyte numbers did not increase. Immunoglobulin levels were normal by day 50. IgE levels were grossly elevated as immunological reconstitution occurred, probably due to T lymphocyte dysregulation. C1q levels became normal. Unusually the IgM isohaemagglutinins showed a higher anti-B than anti-A titre (table 75 ). Competent antibody was present by day 70 as indicated by isohaemagglutinins and the ability of her serum to neutralise her endemic pseudomonas. None of the pseudomonas inhibition studies were performed while she was receiving systemic antibiotics. Schick tests were unreactive i.e. normal, after 2 doses of diphtheria toxoid and cutaneous delayed hypersensitivity to candida was present at day 120. In-vitro T lymphocyte responses to PHA, allogeneic cells and candida immunogen are shown in fig. 18 .

By day 100 after grafting from this unrelated donor there was considerable T lymphocyte function and delayed hypersensitivity to candida was present with normal quantitative and qualitative immunoglobulin levels. This immune function did not persist. Immunoglobulin levels and isohaemagglutinins fell from day 130 but Schick testing remained unreactive and cutaneous delayed hypersensitivity persisted. T cell function suddenly declined and the infant lost weight, developing diarrhoea and chest infections. The graft failed completely by

Table 74a

L.S. CELLULAR RECONSTITUTION

| | Lymphocytes $\times 10^9/l$ | T lymphocytes $\times 10^9/l$ | B lymphocytes $\times 10^9/l$ |
|---|---|---|---|
| pregraft | 0.37 (n=8) | 0.026 (n=4) | 0.268 (n=2) |
| days post graft | | | |
| +35 | 0.30 | 0.08 | 0.12 |
| +50 | 0.48 | 0.02 | 0.07 |
| +70 | 0.69 | 0.35 | 0.05 |
| +100 | 2.4 | 1.98 | 0.07 |
| +130 | 0.48 | 0.19 | 0 |
| +170 | 0.70 | 0.39 | 0.06 |
| +200 | 0.36 | 0.15 | 0.03 |
| +225 | 0.46 | 0.24 | |
| +235 | 1.1 | 0.76 | 0 |
| NR | 2.8–7.65 | 0.18–1.0 | 0.1–1.0 |

Table 74b

L.S. IMMUNOGLOBULIN AND COMPLEMENT RECONSTITUTION

| | Immunoglobulins | | | | |
|---|---|---|---|---|---|
| | gms/l | | | I.U. | |
| | G | A | M | E | C1q % of normal |
| pregraft | 1.4 | .02 | ND | 34 | 35% |
| days post graft | | | | | |
| +35 | 2.1 | 0.2 | 1.5 | 1700 | |
| +50 | 8.5 | 1.1 | 3.0 | 2900 | |
| +70 | 10.2 | 0.8 | 1.4 | 260 | |
| +100 | 12.1 | 0.5 | 0.8 | | |
| +130 | 8.6 | 0.5 | 0.4 | 200 | 105% |
| +170 | 5.6 | 0.5 | 0.3 | 170 | |
| +200 | 5.6 | 0.5 | 0.3 | 200 | |
| +225 | 5.0 | 0.5 | 0.3 | | |
| +235 | 4.8 | 0.4 | 0.3 | | |
| NR | 3–10 | 0.3–0.9 | 0.5–2 | 30 | 100% |

ND – not detected NR normal range

Table 75

L.S. QUALITATIVE ANTIBODY TESTS POST GRAFT AND DELAYED HYPERSENSITIVITY (DHS) TO CANDIDA

| days post graft | anti-A titre | anti-B titre | pseudomonas neutralisation titre | Schick (Dip. toxoid given @ 100 and 170 days) | DHS to candida |
|---|---|---|---|---|---|
| pre-graft | 0 | 0 | none | ND | negative x 2 |
| +50 | | | 1/8 | | |
| +70 | 1/2 | 1/64 | 1/64 | | |
| +120 | 1/2 | 1/32 | | | 7mm |
| +170 | 1/4 | 1/8 | | | 12 mm |
| +190 | 1/2 | 1/4 | 1/64 | no reaction | 10 mm |
| +220 | 1/2 | 1/16 | | no reaction | 7 mm |
| +235 | 0 | 1/8 | | | |
| NR | 1/8 | 1/16 | - | no reaction | reactive |

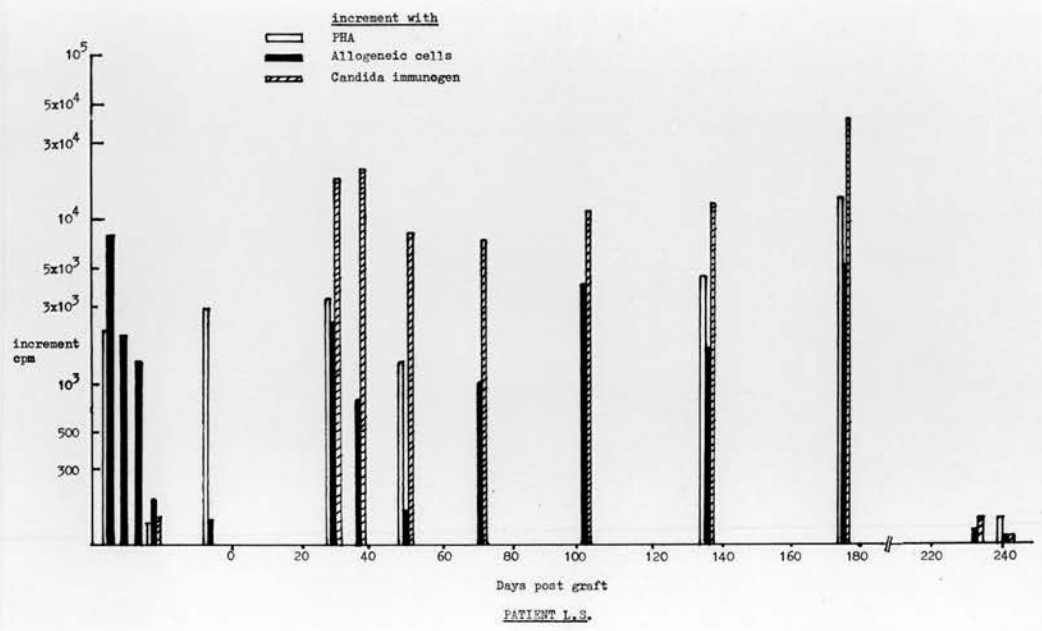
NR - normal range

ND - not done

PATIENT L.S. Fig. 18

RECOVERY OF T LYMPHOCYTE FUNCTION

Increments to PHA, allogeneic cells and candida immunogen



day 240.

Classical rejection had not occurred as the donor was still compatible (table 68). There was no evidence of immune suppression caused by GVHD. Dietary deficiency seemed most unlikely as did a gross metabolic cause. Could the micro-environment have been inimical to the unrelated stem cells which were perhaps unable to co-operate with those of the recipient? A few unrelated grafts have been successful so non-family stem cells can proliferate in an unrelated recipient. Reconstitution with fresh yoghurt between days 140-150 and her removal from isolation did not seem associated with immune decline. Plasma inhibitors were present on day 149 when her plasma suppressed normal third party sheep red cell rosettes by a factor of three. Her own sheep red cell rosetting T lymphocytes increased from 0.39 to 0.48 /l with in-vitro thymosin at that time so her plasma inhibitor was not excess thymic hormone. Because no thymus had appeared on chest x-ray one possibility was that she had outgrown her own supply of thymosin, but the administration of parenteral thymosin (1 mg/kg/day) for two weeks was without effect. The infused erythroblasts could have given an undetected passive red cell graft supplying ADA which would persist only for the 120 day life span of erythrocytes. If subsequent top-up transfusions were insufficient to supply enough ADA, a toxic suppression of graft would have ensued. However deoxyadenosine did not reappear in the urine nor did deoxynucleosides reappear in the red cells, which is against such a toxic suppression of the graft. Whether reticulo-endothelial cells, monocytes and macrophages are engrafted in SCID is not known and it may be that there was imbalance at this level in the marrow stroma. Recent evidence suggests that monocyte grafts may not be permanent (Kersey 1980) so only a transient marrow stroma improvement is possible in this patient, but there is no real explanation of the failure of this transplant.

There is no doubt that two of these seven children have prolonged effective transplants and that these two children are leading a normal life. Only one of the seven had a compatible sibling, a situation that usually gives a 65% success rate, and

two had a compatible parent, a circumstance giving about a 40% chance of success (Bortin and Rimm 1977). Other less well matched grafts have success rates of 5-20%. No other therapy has any real success in treating SCID although occasional claims are made for fetal tissue grafts. Red cell transfusions to supply ADA in those with coincident ADA deficiency may be effective but this usually requires very early diagnosis and institution of transfusion therapy. Until other enzyme deficiencies are identified and enzyme therapy is more effective, marrow transplantation remains the treatment of choice.

MANAGEMENT OF INFANTS WITH SCID

Once the infant is diagnosed as having SCID, the tissue types and mixed lymphocyte culture (MLC) responses of all appropriate family members should be ascertained as soon as possible. Tissue typing should be available within a few days as the tests take one day to perform and specimens can travel by overnight post. An HLA identical sibling has a 98% chance of being acceptable as a donor. The MLC takes 6 days and is not as widely available as tissue typing. The earlier the infant is assessed and appropriate isolation and decontamination established the better, so the child should be transferred as soon as possible to a referral centre where the marrow graft may be carried out. Delay in referring the infant is engendered by the improvement that occurs in hospital with appropriate antibiotics, dietary changes and fluid therapy. A lack of realisation that, once deteriorating, these infants die rapidly and that grafting becomes more difficult, coupled with an understandable reluctance to send the family many miles from home all contribute to the delay.

The referral centre can organise appropriate further investigations with appropriate blood replacement and family studies can be performed at the same time as the MLC. Precise definition of the nature of the SCID in their infant in association with the family studies may give information important in the genetic counselling of the parents which can be undertaken either at the referral centre or more locally. In addition, having met the staff and seen the centre, the parents may feel less anxious about subsequent infants requiring a

"germfree" delivery or early neonatal assessment at the same centre. After seeing and assessing the child fully, the staff are in a position to discuss the infant's problems and the therapeutic options with the parents.

Isolation and decontamination

The infant should be nursed in protective isolation. A clean cubicle with sterile linen and attendants wearing protective clothing and taking proper barrier nursing precautions are necessary. This does not preclude the trained parent. During the first few days, microbiological assessments and decontamination take place and if a plastic isolator is to be used, this can be discussed with parents who then familiarise themselves with its use, so they can continue to play a full part in the care of their infant. Transfer to the isolator would occur when decontamination is satisfactorily established, usually around the same time as the MLC results become available. The benefits of the isolators were already discussed on page 81.

Infants have shown no long term emotional or physical problems as a result of isolation although only six isolated for more than one year have been reported. They tended to be placid and their maturational development was ahead of their learned development but all showed quick catch-up after release from isolation.

I would not recommend using an isolator however until a therapeutic programme has been decided upon as it is not as easy to remove a well though immunoincompetent infant from an isolator as might be imagined.

NEOCON (table 20 ) would be appropriate as antimicrobial decontamination but with increased antifungal agents. Septrin should be avoided until ADA deficiency has been excluded as profound neutropenia is associated with these two circumstances coinciding (EBMT 1980).

The question of bacterial contamination and GVHD (page 43 ) remains open in SCID. Swabs in transport medium from the scalp, ears, nose, throat, axillae, umbilicus, groins, vagina and toe clefts as well as specimens of sputum, urine and stool should be taken before decontamination is commenced. Viral cultures should be sent from the throat, and sputum, nasopharyngeal

aspirate, stool and urine also examined for viruses by electron microscopy or direct immunofluorescence as appropriate. Further microbiological surveillance should follow that outlined in table 50 with additional viral studies on account of the defect in cell mediated immunity.

Humoral replacement therapy

The content of normal human immunoglobulin (NHI) and plasma is as follows

IMMUNOGLOBULIN PREPARATIONS (gms/l)

| | IgG | IgA | IgM |
|---|---|---|---|
| NHI | 150 | 2-6 | 1 |
| Plasma | 5-14 | 0.5-3.0 | 0.5-2 |
| Triple strength plasma | 15-42 | 1.5-9 | 1.5-6 |

The normal synthetic rate of IgG of 34 mg/kg/day would require 0.23 mls/kg/day of NHI (150 mg/ml) by the intramuscular route. If the usual dose of 50 mg/kg/week of NHI is given to a 4 kg infant, the volume is an acceptable 1.3 mls but this replacement is only 20% of what would be synthesised normally in an older child and the standard dose of 25 mg/kg/week is a mere 10%. NHI supplies little IgA or IgM. To supply IgG in amounts comparable to the normal synthetic rate would require 1.4 mls/kg of NHI by the intramuscular route each week, 45 mls of plasma/kg/week or 14 mls/kg/week of triple strength plasma.

The true amounts required to maintain a satisfactory level would be greater as catabolism of IgG increases at higher serum levels. In addition the infant is hypercatabolic owing to infection and impaired nutrition. The sodium content and volume of plasma are other problems. It is not surprising that it is most unusual to achieve serum levels in excess of 2.5 gms/l of IgG with 50 mg/kg/week of intramuscular NHI. High dose intravenous NHI (Ochs et al 1980) and the comparable subcutaneous preparation (Berger et al 1980) should be evaluated and the indwelling subcutaneous needle should not lead to an increased risk of infection. Specific high-titre herpes simplex, varicella or pseudomonas immunoglobulin may have a limited value. Immunoglobulin replacement therapy has not a great role in treating already present infection but may help phagocytosis although

plasma would probably do this better. The low CIq in many SCID patients does not make a great in-vivo difference as the alternate pathway of complement activation is probably intact.

Infections

The majority of bacterial infections are due to common pathogens, predominantly gram-negative bacilli but pneumococci cause some morbidity and if present are an indication for continuous penicillin. Staph. aureus is not a major problem but fungal infections are common. Topical antifungal agents are necessary and the newer absorbable antifungal agents such as ketoconazole which also has some immunostimulatory action need evaluation. However, an infecting organism must not be considered eliminated until there is reconstitution of immunity. If a live immunisation has been given to the infant it must be assumed that the organism is still present and appropriate therapy instituted if possible. One infant who received BCG at birth died of systemic BCG disease after 6 months, the diagnosis being delayed by gentamicin partially suppressing the organism. Diagnosis of infection must be by culture, antigen detection, or microscopy. Specific antibody is not formed but vacuolation of peripheral lymphocytes (best observed in a cytocentrifuge preparation) occurs with some viral infections.

Interstitial pneumonitis may or may not be due to pneumocystis carinii. Lung aspiration using a 21-gauge needle has slight dangers but may be useful. Open lung biopsy is a major undertaking. If due to pneumocystis, response to high dose co-trimoxazole usually occurs within 48 hours. Unfortunately some patients with pneumocystis do not respond, some cases are due to bacteria and some are viral in origin. Failure to respond after 48 hours of high dose Septrin should be followed by lung aspiration with gram and giemsa staining and both bacterial and viral culture of the aspirate. Electron microscopy may also be useful, as may direct immunofluorescent studies for viral antigen on cells present in lung or nasopharyngeal aspirates.

Candida spp. in properly taken urine specimens or in a suprapubic aspirate makes the use of systemic antifungal agents likely but there is not an automatic necessity to continue these daily for six weeks. Some units give amphotericin B for

two days each week, and are prepared to commence this on the slightest supposition of non-superficial candida infection. With any suggestion of herpes or cytomegalovirus infection, acyclovir should be used. Interferon or irradiated parental lymphocytes may be useful in other viral infections. However any remedies can only be of any practical value if cure of the basic problem is under way.

Prophylactic septrin may have disadvantages as two of six SCID infants in this series developed severe neutropenia. In the ADA deficient infant this was definitely caused by co-trimoxazole, and this is now recognised as a relatively common circumstance in infants with ADA deficiency (EBMT 1980). Folinic acid might have protected the marrow but would it also protect pneumocystis carinii? Exposure to pneumocystis before the age of one year is likely as 6 of 14 normal children between seven and twelve months of age had antibody to the organism (Meuwissen et al 1977).

Supportive care

Nursing care of these infants must be meticulous and paediatric nurses are the only ones appropriate. There is no place for these children in a general transplant unit as the nursing problems are too specialised (Birtwhistle 1977). Skilled physiotherapy is probably more important than antibiotics in keeping the lung fields clear (Young 1978).

Adequate oral intake of nutrition is unusual and diarrhoea with malabsorption reduces the energy supply further. Supplemental intravenous feeding is usually required. Central catheters, inserted with full sterile precautions should be used for intravenous feeding but infants are too small to allow silastic 'Hickman' catheters to be used. The standard size are suitable for intravenous feeding, as well as giving and taking blood. There is a need for an infant catheter which can be used in this way without the narrow diameter leading to blocking of the lumen.

Taking blood specimens needs to be properly organised. Micro or semi-micro laboratory facilities are essential and it is necessary to speak personally with laboratory staff to ensure that "inadequate samples" are in fact adequate.

Communication is required whereby one laboratory will pass the remainder of a sample on to another. Quantities quoted in general hospital pathology services handbooks are impossibly large.

Blood products must be irradiated to 1500 rads before transfusion (except for the transplant). This prevents stem cells in the transfusion proliferating and mounting a GVH reaction. Washing of red cells is useless and though nitrogen freezing may be effective, radiation is more certain.

The family

The care of the infant includes care of the family. From the pleasure of a new baby the parents over a few weeks realise that the infant is unwell and seek medical advice on a number of occasions. Not surprisingly they are at first reassured and the candidiasis is treated. The infant's respiratory affliction is treated and the loose stools ascribed to antibiotics or the respiratory affliction. By 3 months the infant is failing to thrive, miserable and has skin rashes. Finally the diagnosis is made and the family face the unlikely possibility of cure. There is guilt about not having taken the infant back earlier to the doctor and "insisted on him doing something", accompanied by anger at the diagnosis having been delayed. Much of this passes once the rarity and the implications of the diagnosis are explained.

For transplantation, the infant plus mother often have to move many miles from home with loss of the support of family and friends. Mother will be in continual close contact with her sick child for many weeks and may feel guilty if she leaves him. Other children at home will suffer from mother's absence and father may not be able to take unlimited time away from work. The "therapeutic community" of the resident parents' accommodation may not be entirely helpful. Great stress will be placed on the marriage and parents should very definitely not spend unlimited time at the transplant centre, but be encouraged to be together at home whenever possible. No matter the outcome of the transplant, the family itself has a future which we must do our best to safeguard.

## GERMFREE DELIVERY

### Introduction

The genetics of SCID will have been explained to the parents as best as possible and the implications of a further pregnancy will have been discussed at length. In the light of this, many parents will elect to have further children and therefore a germfree delivery will have to be considered, even if only to be discarded as a possible option. Techniques to obtain germfree animals have been available since the first germfree delivery of a goat in 1913. The first delivery of an axenic human infant was in 1967 (Alpert et al 1969) and the first such delivery performed on account of possible immune deficiency was reported by Barnes et al (1968).

It is essential to think clearly about the implications of a germfree delivery performed on account of potential immune deficiency in the infant. An affected axenic infant maintained in a germfree environment but without a curative treatment programme is a difficult moral problem for every member of the medical and nursing staff. To discontinue germfree care in this situation is not as easy as it seems. Prior discussions and decisions made by the parents may no longer be valid. Texas Children's Hospital, Houston are now in their eighth year of caring for a child with SCID who has no bone marrow donor and remains very well both physically and psychologically in a plastic isolator. Although his team felt that "presented with a similar circumstance, our team would assume a similar role in the care of another child" and that "sterile delivery may assume more importance in the future" (Malinak et al 1973), it is noteworthy that they have performed no similar deliveries in the past eight years.

Germfree delivery is only appropriate when other alternatives have been considered, if only to be rejected. If the combined immune deficiency is of sex-linked inheritance, then sexing of the fetus and abortion of the males is possible however distasteful. With associated ADA or other enzyme deficiency, amniocentesis followed by fibroblast culture and determination of enzyme activity may be able to distinguish between affected and unaffected infants. Variation of ADA activity in different tissues and the difficulty of distinguishing

the affected from the carrier infant complicates the results. Immunological assessment by fetal blood sampling will be possible in the future.

Given that the parents wish the infant delivered, the final benefits of a germfree delivery are speculative. However, an affected infant will return infected by three months and should a transplant be possible, it would seem sensible to have the child in good condition and proceed as soon as possible. Germfree delivery implies having the facilities to continue this standard of isolation subsequent to the delivery. To suggest that an affected infant remain in an isolator while the parents have another child who might be a marrow donor is more than mischievous.

Any form of germfree delivery involves separation of the infant from its parents. This is not a real problem as the family have already lost a child with immune deficiency and although there is a better than even chance that the new infant will be normal, it is likely that some degree of mourning for the new baby has already occurred before the birth. I do not believe that the week necessary for the initial tests will blight the parents' relationship with the new infant. The infant needs cared for and parents who are familiar with isolation nursing techniques can perform the care. This can be taught before labour commences and if the previous infant was cared for in the same hospital, the staff and routines will be familiar to both parents. Mother can also see and touch the baby transiently between delivery and isolation without a host of pathogens being transferred to the child.

Three deliveries

The choice lies between caesarian section and vaginal delivery. Caesarian section can be pre-arranged and the incision and delivery made through the drape in a closed sterile surgical isolator. The newborn is then passed through into a connecting sterile cot isolator thus maintaining a truly germfree state. Despite this, I am not in favour of caesarian section. Apart from the inherent dangers of the operation there are two major disadvantages. Statistics favour the child being unaffected by SCID and if active resuscitation of the infant is

required, this is still impractical within an isolator. Barnes et al (1968) reported how they had difficulty intubating in their isolator and despite improved models of isolator this is too great a price to pay.

Delivery by the vaginal route results in greater exposure to the environment but adequate local decontamination can be achieved using iodinated compounds (e.g. Betadine) which also have anti-viral activity. An appropriately secured plastic sheet should prevent faeces soiling the child. There is little point in giving mother oral non-absorbable antibiotics and if the birth canal is adequately decontaminated such antibiotics should not be necessary for the child. Vaginal delivery allows such resuscitation as necessary in optimal conditions and mother to touch and see the infant before he is confined in a controlled environment.

Three such deliveries were undertaken using techniques described in appendix 2 . Our obstetric unit was in the main Westminster Hospital, 800 yards from the Children's Hospital. There were no paediatric staff continuously in the obstetric unit and thus the staff responsible for the infant had to be summoned at the appropriate time. A senior paediatric registrar and a nurse were sufficient, the required equipment being already prepared and in place.

A specially cleaned, fumigated and sealed Vickers 59 incubator was used because the humidity port exactly fitted a Microflow 0.3$\mu$ LF 40 air filter. Filtered air from a 5 cfm mains/battery blower gave about 20 air changes/hour, which could be increased if the incubator ports were opened. Because this air flow will cool the infant, the room and therefore the air, must be at the required incubator temperature.

The infants were delivered vaginally without surgical interference but with the topical application of copious amounts of local Betadine. They were received onto a sterile sheet in which they were wrapped and dried. No resuscitation was required but the infants were observed for a minute on a Resuscitaire trolley covered in sterile drapes, shown to the parents and then placed in the prepared incubator. The incubator, still with positive pressure filtered air, was transferred by ambulance

to Westminster Children's Hospital. The infants remained in this incubator in a specially cleaned cubicle until immunological tests were reported. All attending staff wore masks, gowns, gloves, hats and overshoes and all supplies used were sterile.

Surface and orifice decontamination using Hibiscrub (4% chlorhexidine in detergent) topically and oral sprays of 0.02% aqueous chlorhexidine were used from age 2 hours, but no antibiotics administered. The infants were fed 5% dextrose initially followed by Gold-cap SMA. When the mother's milk came in, this was pasteurised and given within two hours of collection.

Placental blood, collected by cannulating the 'umbilical' vein was used for the initial investigations. Two infants were normal and one was affected by SCID. Although there was no obvious family donor for this infant a therapeutic programme had been previously discussed, that of attempting to induce tolerance in father and render his lymphocytes unresponsive to the non-shared HLA loci of the child. Since there had been encouraging experimental results with this technique, the infant was transferred to an isolator tent age 4 weeks. The outcome is awaited. The infants who were normal were transferred to a conventional environment or home depending on circumstances.

Results

Table 76 shows the microbiological results of these delivery techniques. The infants acquired staph. epidermidis readily but faecal organisms, strep. faecalis and anaerobes, appeared after the third day. Infant AQ was contaminated with faeces at delivery, but it is likely that the peptostreptococci were acquired in the birth canal. The presence of the other organisms indicates some deficiency in our cubicle/incubator protective care. The pasteurised breast milk (given from day 3) may have been a source of organisms. Should the maintenance of a totally axenic state in SCID be vital, then caesarian section with a sterile surgical isolator will have to be reconsidered.

Table 77 shows the cord blood results from the three infants. DF and AQ were normal infants; AN suffered severe

Table 76

MICROBIOLOGICAL RESULTS FOLLOWING VAGINAL DELIVERY

| Patient | Age | Sites cultured | No. of sterile cultures | Organisms isolated from non-sterile cultures |
|---|---|---|---|---|
| DF | 3 hrs | 8 | 8 | - |
| | 28 hrs | 5 | 3 | staph. epidermidis (light growth) |
| | 76 hrs | 11 | 2 | staph. epidermidis (light growth) |
| | 8 days | 9 | 0 | staph. epidermidis (light growth) |
| | | | | diphtheroids ) |
| | | | | coliforms ) in stool |
| | | | | strep. faecalis) |
| AQ | 2 hrs | 3 | 3 | - |
| | 24 hrs | 4 | 3 | clostridia spp. ) in stool |
| | | | | peptostreptococci) |
| AN | 4 hrs | 12 | 12 | - |
| | 7 days | 6 | 1 | staph. epidermidis (light growth) |
| | | | | micrococci (light growth) |
| | 10 days | 7 | 0 | staph. epidermidis |
| | | | | strep. faecalis ) |
| | | | | anaerobes ) in stool |

Table 77

CORD BLOOD RESULTS FROM THREE INFANTS DELIVERED WITH BARRIER PRECAUTIONS

| | DF | AQ | AN** | Normal ranges |
|---|---|---|---|---|
| Lymphocytes x $10^9$/l | 9.06 | 9.15 | 1.26 | 2.3–9.0 |
| T lymphocytes x$10^9$/l | 0.91 | 3.3 | 0.34 | 0.6–4.0 |
| B lymphocytes x $10^9$/l | 4.70 | 2.75 | 0.33 | 0.22–2.5 |
| *increments over control (c.p.m.) | | | | |
| to PHA | 50,000 | ND | 0 | more than 9000 |
| allogeneic cells | 9,800 | ND | 0 | more than 9000 |
| Immunoglobulins (gms/l) | | | | |
| G | 5.2 | 8.6 | 4.4 | 7.5–18 |
| A | 0.5 | $<$0.05 | 0.2 | $<$0.1 |
| M | 0.2 | 0.07 | 0.5 | 0.1–0.2 |
| Outcome | Normal infant | Normal infant | SCID | |

* results in autologous plasma; results were similar in AB plasma

** increments following in-vitro SK-SD, and Concanavalin-A were also very low

ND – not done

combined immune deficiency. The T lymphocyte function was the most useful investigation in making or refuting the early diagnosis of SCID.

Summary

A simple form of decontaminated vaginal delivery was used in three instances where the unborn infant was at risk of having combined immune deficiency. The delivery was effected satisfactorily by the vaginal route but the use of a specially cleaned incubator with sterile positive pressure air, sterile supplies and attendants wearing protective clothing did not prevent the infants becoming colonised during the first 7 days by which time the immune status of the infant had been ascertained. However no antimicrobial agents were given to either mother or infant and pasteurised breast milk was given to the infants. More stringent measures need to be adopted if the axenic state is thought to confer additional benefit, but this will increase the likelihood of having to maintain an affected child in a gnotobiotic environment without a therapeutic programme. All these measures will be unnecessary for three-quarters of at-risk infants will be unaffected by SCID.

# SEVERE APLASTIC ANAEMIA IN TEN CHILDREN

## Introduction

Severe aplastic anaemia is characterised by marked peripheral blood pancytopenia and bone marrow hypoplasia. There is anaemia, a reticulocyte count of less than 1% or less than $10 \times 10^9$ reticulocytes/litre, a platelet count of less than $20 \times 10^9$/l and more than 60% of the bone marrow cells are of the non-myeloid series. Excluded from the definition are selective cytopenias such as pure thrombocytopenia or the erythropenia of Diamond and Blackfan (1938), the hereditary anaemias such as that originally described and reviewed by Fanconi (1967), paroxysmal nocturnal haemoglobinuria, myelophthisic conditions such as osteopetrosis and the effects of cytotoxic drugs or systemic illness.

## Incidence and aetiology

In 1978 the Registrar General recorded 255 deaths from aplastic anaemia in England and Wales, of whom 17 were children. About 8% of cases are known to be drug-induced, this being either a direct toxic effect or an idiosyncratic response by a susceptible bone marrow. A further 8% of cases follow a hepatitis, the severity of which is unrelated to the ensuing aplasia. Most such patients are aged less than twenty years and almost all such aplasias are severe. Opinions differ as to whether the aplasia is a direct consequence of the viral infection or the result of an immunological process engendered by the virus.

The cause of 75% of severe aplasias is unknown. There is evidence to favour abnormalities of the marrow, the marrow bed and the lymphoid system. Mice with hereditary aplasia and an abnormal marrow stroma cannot be reconstituted by marrow grafting yet their apparently ineffective marrow will effectively populate the marrow cavities of mice with hereditary stem cell defects (McCullough et al 1965). Rat femur, irradiated locally to 4000 rads will only transiently support the ingrowth of new marrow (Knospe and Crosby 1971). Osteopetrosis can be cured by spleen and marrow transplantation in mice (Walker 1975) and, at least temporarily, in humans (Coccia et al 1980) indicating a close relationship between bone marrow stem cells and the

osteoclast component of bone. The occasional failure of a human syngeneic marrow graft in aplasia may be attributed to an abnormal marrow bed, acquired as a result of unknown toxic processes which may still be operative at the time of grafting.

An immunological cause is suggested by the increased lymphoid component of most aplastic marrow. A failed syngeneic marrow transplant may engraft at a second attempt when immunosuppression is used (Royal Marsden Hospital 1977). Occasionally autologous reconstitution is seen following immunosuppression (Baran et al 1976). In-vitro cultures of aplastic marrow give granulocyte colony or cluster formation in soft agar only when marrow lymphocytes are removed from the preparation (Ascensao et al 1976) and these same aplastic marrow lymphocytes will inhibit colony formation by cultured normal marrow. This effect is particularly evident with added T lymphocytes bearing gamma $F_C$ receptors, so called suppressor cells. (Bacigalupo 1980). The immunological imbalance associated with GVHD (in which excess suppressor cells are often present) has also given rise to aplasia (Park et al 1973; O'Reilly et al 1976).

Natural history

The natural history of aplastic anaemia is variable but table 78 summarises a number of reports of patients with aplasia. The best prognosis quoted is 50% survival to 15 months from diagnosis. If less severe cases have been included, or if some received steroids and/or androgens and if these are helpful, then survival data here is optimistic. If these data are accepted at face value, the survival rate of the quoted series reported since 1968 (excepting Camitta et al who only reported post-hepatitis aplasia) is 35% overall at best and 30% for children. A more likely outcome in severe aplasia is 20% or less alive at 1 year (Feig 1978; Gluckman et al 1980).

Some patients with severe aplasia will survive when given only supportive care. Attempts to identify those most likely to survive have given rise to a number of prognostic formulae, based on the initial presentation data. Table 79 shows five prognostic formulae, and table 80 compares the results of four formulae when applied to the Westminster Children's Hospital

Table 78

APLASTIC ANAEMIA - NATURAL HISTORY

| Author | Date | No. of patients | Age group | Outcome |
|---|---|---|---|---|
| Wolff et al | 1957 | 334 | 25% children | 66% died (30% lost to follow up) |
| Shahidi and Diamond | 1959 | 40 | children | 90% died |
| Nora and Fernbach | 1969 | 37 | children | 78% died |
| Heyn et al | 1969 | 33 | children | 48% died (39% dead in 3 months) |
| Li et al * | 1972 | 58 | children | 70% died |
| Lewis | 1965 | 50 | | 70% died (50% dead in 15 months) |
| Vincent and De Gruchy | 1967 | 43 | | 63% died within 1 year |
| Davis and Rubin* | 1972 | 25 | | 80% died |
| Camitta et al | 1974 | 80 | | 87% died (hepatitis aplasia) |
| Lynch et al | 1975 | 99 | | 43% dead by 4 months |
| Tso et al | 1977 | 129 | | 62% died by 1 year (47% by 6 months) |
| Mir and Geary | 1980 | 147 | | 71% died |

* the majority received androgens

Table 79

PROGNOSTIC FORMULAE IN APLASTIC ANAEMIA

| Author | Date | Formula indicating bad prognosis |
|---|---|---|
| Lewis | 1965 | platelets $<20,000/\mu l$;<br>neutrophils $<100/\mu l$ |
| Camitta et al | 1976 | platelets $<20,000/\mu l$;<br>neutrophils $<500/\mu l$;<br>reticulocytes $<1\%$; marrow $>30\%$ non-myeloid cells |
| Lohrmann et al | 1976 | reticulocytes $<10,000/\ l$ (corrected) |
| Mathé and Schwarzenberg | 1977 | low lymphocyte count; low number of CFU-C |
| Lynch et al | 1975 | C= −0.01796 B (B=onset with bleeding, if yes = 0, if no = 1)<br>+0.01272 A (S=sex; female =1, male = 2)<br>−0.00008 OFV (months between onset and clinic visit)<br>−0.00359 R (R=corrected initial reticulocyte count per cent)<br>−0.000002 N (N=initial neutrophil count/cumm)<br>−0.00018 P (P=initial platelet count in thousands/cumm)<br>+0.00046 NM (NM=percent of non-myeloid cells in initial marrow)<br>If C $>0.035$ the prognosis is probably poor) |

Conversion to SI units

10,000/cumm = $10,000/\mu l$ = $10 \times 10^9/l$

Table 80

FORMULAE USING DATA ON FIRST PRESENTATION

| Patient | Lewis | Camitta | Lohrmann | Lynch | Final Outcome |
|---|---|---|---|---|---|
| JW | G | G | G | G | Grafted - well |
| CT | ? | G* | X | X | Died of infection |
| MB | ? | X | G | X | Grafted, no take, died of infection |
| PM (adult) | G | G* | X | G* | Grafted, died of infection |
| AD | ? | ?* | X | G* | No response to ALG died pre-graft |
| LP | G | G | G | G | Grafted. Died of haemorrhage |
| SH | ? | X | X | X | Died of haemorrhage |
| AU | ? | ? | X | X | Grafted - well |
| LA | ? | ? | G | X | Grafted. No take. Died of haemorrhage |

G = relatively good prognosis

? = uncertain

X = relatively poor prognosis

* when recalculated, using data on admission to Westminster Hospitals, these patients were now of poor prognosis

patients with aplastic anaemia. In five of nine patients the formulae disagreed, the formula of Lohrmann et al (1976) being the most dissimilar. However what is more unsatisfactory is that when the prognosis was recalculated using the data at admission to the Westminster Hospitals, three of nine patients had changed their prognosis, all from a relatively good to relatively poor prognosis. The patients are a selected group in that those doing well would have been less likely to have been referred. No patient changed to the good prognosis group. The Lohrmann prognosis did not change; the others changed to agree with Lohrmann. It seems unlikely that a good prognosis should become poor during a hoped-for spontaneous recovery. Lynch et al (1976) found 8% of those surviving 6 months had been originally in the bad prognosis group, but in our hands, Lohrmann's formula seemed the most reliable.

Treatment

Apart from bone marrow transplantation, treatment with steroids or immunosuppression is available. Androgens are said to take 3 months to produce an effect and marrow improvement may be an epiphenomenon. Matched patient trials of androgens in aplasia have shown no significant benefit (Lynch et al 1976) yet there are certain patients who do benefit from androgens and relapse when they are discontinued. The mode of action is unknown although androgens increase erythropoietin production in the isolated perfused dog kidney (Paulo et al 1974). However, there is no evidence that aplastic anaemia is associated with deficiency of or insensitivity to erythropoietin. Of the androgens available norethandrolone 0.5-1 mg/kg/day is perhaps the most effective (Speck et al 1980). There is a definite incidence of hepatotoxicity (Tso et al 1977) and many patients develop abnormal bromsulphalein excretion or liver enzymes (Sanchez-Medal et al 1969; Corneo et al 1971). Lithium carbonate may increase the peripheral polymorph count (Garrett et al 1977) and marrow granulocyte colonies but does not affect the platelet count. Although lithium has no real place in the management of aplasia, it may accelerate marrow recovery after grafting and thereby slightly reduce the period of severe neutropenia.

Immunosuppression has given responses in aplasia. Apart from cyclophosphamide used as pre-transplant conditioning, the two most used immunosuppressants have been steroids and anti-lymphocyte globulin (ALG). Marmont (1979), using methyl-prednisolone 20 mg/kg/day for 21 days, reported a 46% complete remission rate in 26 patients with severe aplasia. He reported no difficulty withdrawing the steroids and those patients with compatible donors who did not respond were transplanted. Marrow cultures from the responding patients showed only clusters whereas cultures after successful grafting gave normal colonies. Speck et al (1977) gave ALG with or without a one haplotype identical marrow transplant. Overall survival in both groups was 55% at 1 year with 41% showing sustained haematological improvement. No graft took so there was no GVHD, but 70% of the patients developed serum sickness following the equine globulin. Combinations of equine and rabbit ALG have given a similar response rate. (Bacigalupo 1980).

Marrow grafting is the curative treatment of choice. Transplantation from a compatible sibling before the patient has received any transfusions has given an impressive 75% two year survival (Storb et al 1980). This can be compared with Seattle, Los Angeles and the European transplant series (table 81 ) where an overall survival of 43% is quoted with 30% of patients suffering GVHD. Unfortunately untransfused aplastic patients are not common. If the value of cyclosporin-A in preventing GVHD in allogeneic transplantation for acute leukaemia (Powles et al 1980) is confirmed in aplastic anaemia, then a major hurdle will have been overcome.

Despite circulating stem cells being sufficient to graft dogs and rodents and give GVHD in infants with SCID, the numbers of stem cells and nature of the human haemopoietic system make this an inadequate graft for a patient with aplasia. A syngeneic graft using leukaphoresis from peripheral blood failed but a marrow graft which supplied twice as many stem cells succeeded (Hershko et al 1979). The intravenous route is the most convenient although intra-peritoneal, intra-arterial and intramedullary routes have been used. Different handling techniques of donor marrow (Barrett et al 1979) confer no

advantage providing the donor marrow is given as soon as possible after harvesting.

The marrow donor is usually an HLA identical, MLC unreactive sibling, although occasionally a parent is suitable. Unlike heart or kidney grafts, ABO compatibility between donor and recipient does not affect the results. An ABO mismatch usually requires the recipient to be plasma exchanged before and possibly after the graft. Sufficient plasmaphoreses are required before the transplant, to reduce the haemolysin titre to 1/4 or less. Donor type red cells are then infused carefully to "mop-up" remaining antibody and the patient transplanted. Occasionally Witansky substance may be used. Following graft-take some ABO incompatibility and a minor haemolytic state may persist for some weeks but this is rarely more than a minor clinical problem and the patient's blood group and iso-haemagglutinins become those of the donor by the third month.

Preconditioning for transplantation

Immunosuppression is required for all but syngeneic grafts which are virtually out-patient procedures with only the recipient's other problems governing the management. The traditional protocol is derived from matched littermate dog data.

| | | |
|---|---|---|
| Day | -6 | Donor buffy coat |
| | -5 | Cyclophosphamide 50 mg/kg |
| | -4 | Cyclophosphamide 50 mg/kg |
| | -3 | Cyclophosphamide 50 mg/kg |
| | -2 | Cyclophosphamide 50 mg/kg |
| | -1 | |
| | 0 | Graft |

Donor buffy coat is not essential but is given to stimulate host reactive cells which when dividing in response to this stimulus will be destroyed by cyclophosphamide. This regimen has proved reasonably effective in patients who have received only a few transfusions. However, blood products from family members or transfusions from more than 10 random donors will sensitise the recipient. The four day cyclophosphamide regimen is then less effective and greater immunosuppression is required, especially if the patient is refractory to random donor platelets as manifest

by a platelet increment of less than 20 x $10^9$/l one hour after infusing $10^{11}$ platelets/sq. m. body surface area.

To reduce the high rejection rate in patients sensitised to their donors, regimens including procarbazine, ALG, TBI and cyclophosphamide were used and although with TBI graft rejection was much less common, many more patients died from infection and GVHD (Gale 1978). For patients both sensitised to their donors and refractory to random platelets, the addition on days 1 to 5 after grafting of donor buffy coat to the standard four day cyclophosphamide preconditioning regimen reduced the rejection rate from 81% to 37% and increased the survival from 25% to 63% of patients without any increase in GVHD (Feig 1978). The intention is to supply additional stem cells to those obtained by marrow harvest but the presence of this large amount of donor antigen and competent granulocytes during the recovery phase may be of relevance.

Total nodal irradiation (including the spleen) with lung shielding attempts to avoid the radiation effects of TBI on the lungs. Early results were promising and graft rejection was much reduced. Kersey (1980) recently reported 20 aplastic children, all of whom had received multiple transfusions, who were successfully engrafted with matched sibling marrow, using 4 days of cyclophosphamide followed by 900 rads total nodal irradiation. 7 of the 20 developed GVHD with 3 deaths, so the problem of GVHD remains. It may be that 900 rads is excessive and less or more limited radiotherapy will prove effective, but this should not affect the incidence of GVHD.

Failure to engraft

Rejection of the transplant is associated with a transplant of less than 3 x $10^8$ nucleated stem cells/kg recipient body weight, and prior sensitisation of the recipient to the donor (Storb et al 1978). Attempts to predict this latter circumstance using the MLC, the relative response index (RRI) of recipient to donor compared to pooled random lymphocytes have not shown any one test to be superior (Storb et al 1977). Lymphocytotoxins in the recipient pre-graft may correlate with failure of engraftment (Gale et al 1978) but others (Gluckmann 1978) believe these are only significant if they persist after the

transplant.

However, all these predictors of graft rejection are becoming less relevant as with the use of total nodal irradiation, busulphan and cyclophosphamide, and more extensive preconditioning before the transplant, graft rejection is becoming less common. With adequate preconditioning graft-take is almost assured but the preconditioning itself must not give unacceptable side effects.

Graft-versus-host disease

Prophylaxis against GVHD is usually given and until the advent of cyclosporin-A, methotrexate was used. In dogs this is highly effective (Storb et al 1970) but there are no controlled human studies. No studies of prophylactic cyclosporin-A in the grafting of aplasia have been reported. Other measures such as incubation of the marrow with ALG or steroids to deplete the infused marrow of T and pre-T lymphocytes are presently under trial as is the use of monoclonal antibody.

Results of marrow transplantation

Some results of transplantation in aplastic anaemia are shown in table 81 . Only a few patients had Fanconi's anaemia which presents it s own special problems. Bortin (1976) presented results reported from 17 centres participating in the International Registry. Storb et al (1978) showed no change in the percentage surviving between 1974 and 1978. The best results are in the only paediatric series (Johnson et al 1976), and these are as good as the results from those who have received no transfusions before grafting. Of 30 patients grafted within 48 hours of receiving their first transfusion of blood products, 75% have survived two years (Storb et al 1980). However there does seem to be a high incidence (36%) of chronic GVHD (Gluckmann 1980). The European groups (Gluckmann et al 1978; Barrett 1979) have more problems with graft rejection than workers in the United States, and overall in Europe 1971-1979 (Gluckmann et al 1980) only 40% survived more than 1 year. Approximately 5% of all patients died early after transplantation. Infection alone killed 13% and infection accompanied by GVHD killed a further 27% of those successfully grafted. GVHD was implicated in 71%

Table 81

MARROW TRANSPLANTS FOR SEVERE APLASTIC ANAEMIA

| Author | No. of patients | No. with graft take | No. with acute GVHD | Survivors |
|---|---|---|---|---|
| Bortin 1976 | 38 | 27 (71%) | ? | 18 (47%) |
| Storb et al 1974 | 24 | 21 (88%) | ? | 11 (46%) |
| Johnston et al 1976 | 22 | 19 (86%) | 3 (16%) | 16 (73%) |
| Gale et al 1978 | 20 | 16 (80%) | 8 (50%) | 7 (35%) |
| Storb et al 1978* | 110 | 76 (69%) | 31 (41%) | 50 (45%) |
| Gluckmann et al 1978 | 25 | 11 (44%) | 8 (72%) | 9 (36%) |
| Barrett 1979 | 23 | 13 (57%) | 6 (46%) | 10 (43%) |
| Gluckmann et al 1980** | 162 | 105 (65%) | 69 (66%) | 69 (42%) |

* includes results of Storb et al (1974) and Johnston et al (1976)

** includes Gluckmann et al (1978) and Barrett (1979)

of the post transplant deaths. Infection was the major cause of death in those whose grafts were rejected. Few patients who survive 150 days after the graft die, but 10% long term survivors are disadvantaged by chronic GVH (Storb et al 1976) a figure which shows signs of rising.

In table 82 , where possible, children are separated from each series. In each report and in the recent report of 162 European patients (Gluckmann et al 1980) patients age less than seventeen do better as regards graft take, incidence of GVHD, and survival. From the natural history (table 78 ) a better survival compared to adults is not a feature of childhood aplasia and both facts give encouragement to the policy of early transplantation in severe aplastic anaemia in childhood.

Fanconi's hypoplastic anaemia

Children suffering Fanconi's syndrome are usually short and have café-au-lait patches, skeletal and renal abnormalities, and characteristically progressive pancytopenia first presenting as bleeding in middle childhood. Over the ensuing years neutropenia and anaemia develop and transfusions are increasingly required. Chromosome breaks, gaps and endo-reduplications increase with time. Most patients die of haemorrhagic problems but 25% develop acute myeloid leukaemia. However there is a spectrum of severity and it may be that the mesenchyme is more affected than other tissues in some patients.

Deficiency of superoxide dismutase allowing free superoxide radicals to persist has been identified in a number of cases and in-vitro culture of Fanconi cells with this enzyme reduces the number of chromosome abberations observed (Nordenson 1977) whereas the addition of very dilute activated cyclophosphamide to cultures of Fanconi cells causes an increased number of chromosome abnormalities (Berger et al 1980). This must be a partial explanation for the severe mucositis that arises following bone marrow transplantation in that the basic defect in DNA repair is intensified by alkylating agents.

With an almost inevitable natural history, a marrow transplant is appropriate but there are difficulties additional to those of grafting in severe aplasia. Timing of the graft is difficult as subjection of the child to a possibly lethal

Table 82

MARROW GRAFTS FOR SEVERE APLASIA

REPORTS OF PATIENTS AGE LESS THAN 17 YEARS

| Author | No. of patients | No. with graft take | No. with GVH | Survivors |
|---|---|---|---|---|
| Bortin 1976 | 22 | 20 (91%) | ? | 13 (59%) |
| Johnson et al 1976 | 22 | 19 (86%) | 3 (16%) | 16 (73%) |
| Barrett 1979 | 13 | 9 (69%) | 2 (22%) | 7 (54%) |

REPORTS WITH PATIENTS AGE 17 OR LESS EXCLUDED

| Author | No. of patients | No. with graft take | No. with GVH | Survivors |
|---|---|---|---|---|
| Bortin 1976 | 16 | 7 (43%) | ? | 5 (31%) |
| Storb et al 1978 | 88 | 55 (62%) | 26 (47%) | 34 (39%) |
| Barrett 1979 | 10 | 4 (40%) | 4 (100%) | 3 (30%) |

procedure when the child seems well has to be balanced against probable increasing sensitisation to blood products and progressive chromosomal abnormalities. Although washed nitrogen frozen red cells will reduce HLA sensitisation, platelet transfusions undo any such benefit. Since no rejection of a sibling transplant has been reported in a Fanconi patient sensitisation may not be a great problem. However a one haplotype identical parental marrow was recently rejected at Westminster. The children are particularly prone to mucosal complications of cyclophosphamide preconditioning and methotrexate GVHD prophylaxis, developing severe mucositis and haemorrhagic cystitis. To use irradiation would be disastrous. They are also at particular risk of severe GVHD, with a 80% mortality rate in those who develop GVHD.

There have been attempts to obviate these complications. Half-dose preconditioning with folinic acid rescue after each dose of methotrexate did not minimise the severe mucositis and three of five patients developed fatal acute GVHD (Gluckmann et al 1980). If there is a progressive cell mediated immune defect (Pedersen et al 1977) then it might be helpful to wait for this state and transplant with minimal preconditioning. Whether grafting early or late will make any difference to the incidence of malignancy remains to be seen, but at least one patient remains well 5 years after his graft (Barrett et al 1977) Table 83 shows the results of transplantation for Fanconi's anaemia, with an apparent overall success rate of 29%, but probably many other grafts have been performed but not reported.

## CLINICAL MARROW TRANSPLANTATION FOR SEVERE APLASIA

Seven children with idiopathic aplastic anaemia and 3 with Fanconi's anaemia were prepared for bone marrow transplantation at Westminster Children's Hospital. Three children died 24 hours before grafting; three showed no evidence of graft-take and subsequently died and four had established grafts, one of whom died of acute GVHD. Three of the seven transplanted remain well at least 24 months later. Details of these transplants are shown in table 84 . The general management and organisation of these transplants is discussed from page 143 . Typical clinical courses following transplantation for aplasia are

Table 83

MARROW GRAFTS IN FANCONI'S ANAEMIA

| Centre | Patients | Alive | Dead | GVHD | Cause of death | |
|---|---|---|---|---|---|---|
| | | | | | GVHD | infection |
| Paris | 5 | 1 | 3 | 5 | 4 | |
| Seattle | 4 | 1 | 3 | 2 | 2 | 1 |
| Westminster | 3 | 1 | 2 | 1 | 1 | 1 |
| Others | 2 | 1 | 1 | 2 | 1 | |
| | 14 | 4 | 9 | 10 | 8 | 2 |

Table 84

## TRANSPLANTS FOR APLASIA AND FANCONI'S ANAEMIA

### Idiopathic aplasia

| Patient | Age | Sex | Size of graft nucleated stem cells/kg | Donor | Outcome |
|---|---|---|---|---|---|
| CT | 4 | M | - | - | Died pregraft (infection) |
| MB | 15 | F | 2.2 | Brother | No take |
| | | | 2.7 | Brother | No take |
| | | | 1.4 | Sister | No take; died of infection |
| JW | 16 | F | 9 | Sister | Proven graft; mild chronic GVHD resolved; now well |
| AD | 15 | F | - | - | Died pregraft ?cyclophosphamide myocarditis |
| LP | 11 | M | 3.8 | Brother | Transient reconstitution |
| | | | 2.7 | Brother | No take; died of haemorrhage |
| LA | 4 | F | 4 | Half-brother | No take; died of haemorrhage |
| AU | 13 | M | 2.7 | Sister | Proven graft - well |

### Fanconi's anaemia

| Patient | Age | Sex | Graft size - nucleated cells x $10^8$/kg | Donor | Outcome |
|---|---|---|---|---|---|
| MR | 14 | M | 6 | Brother | Proven graft - well |
| RR | 14 | M | - | - | Died pregraft - infection |
| KG | 9 | F | 4 | Sister | Died of acute GVHD |

shown in figures 19 to 27 .

Successful engraftment

1) Patient AU

He presented with two months of bruising and one month of tiredness and pallor. He had a haemoglobin of 4.2 gms/dl, leukocytes 2.3 x $10^9$/l, polymorphs 0.6 x $10^9$/l and platelets of 14 x $10^9$/l. His corrected reticulocyte count was 0.4 x $10^4$/l. Despite his presenting haemoglobin and platelet count, he received no transfusions until nine days before commencing cyclophosphamide preconditioning. Washed nitrogen frozen red cells supplied by the Army Medical Centre, Aldershot, were used to minimise exogenous antigenic stimulation. Allogeneic platelets were given three days before cyclophosphamide. His HLA, MLC compatible sister donated marrow and 26 days after grafting female polymorphs appeared in the peripheral blood (Fig. 19 ). He showed a lymphocyte peak on day 8, a feature of female to male grafts (Barrett 1978). Norethandolone 0.5 mg/kg/day was given as his engraftment was rather slow. He discontinued isolation and was discharged home on day 65. He has since remained well and retains a female karyotype in both marrow and peripheral blood chromosome preparations. Further details of his reconstitution are discussed later.

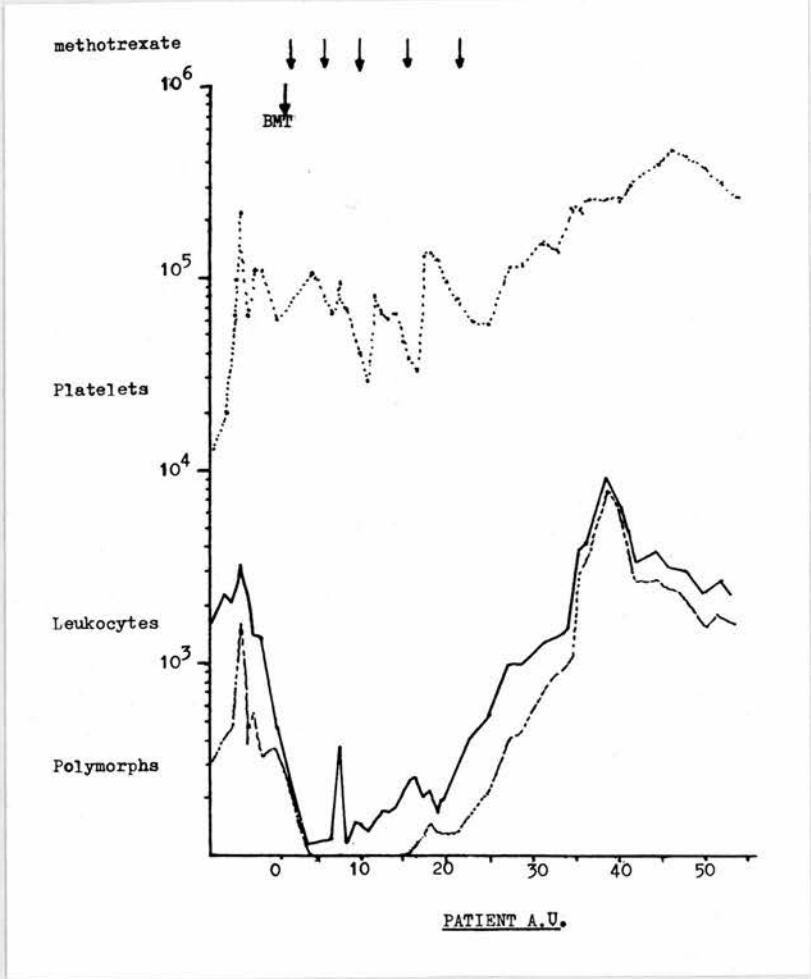
Figure 20 shows his clinical progress, the least complicated of any of the patients. He never developed any rash and bilirubin and alkaline phosphatase levels remained normal. Alanine aminotransferase (ALT) levels were raised between days 15 and 40 days. The sudden rise around day 50 was attributed to norethandrolone, which was discontinued. Fever during the leucocyte nadir between days 6 and 11 was microbiologically unexplained but that between days 42 and 47 was caused by a cryophilic kliebsiella spp., probably acquired from the blood transfusion on day 42. On only three days did he have more than two stools a day.

His relatively smooth course was due to a number of factors; the rapidity of the referral; the courage of the referring paediatrician in not transfusing him; and his initial lack of infection with a polymorph count of 0.6 x $10^9$/l. Prophylactic leukaphoreses given from days 5 to 11 were probably

PATIENT A.U.

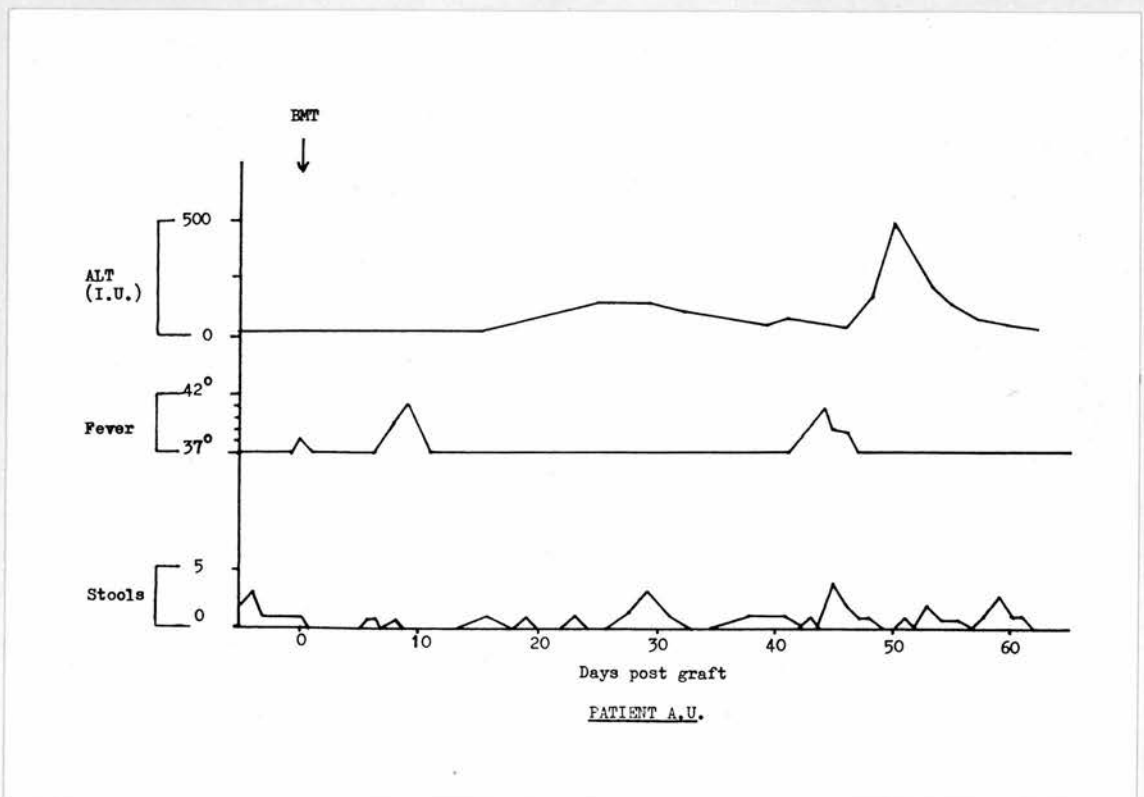
HAEMATOLOGICAL PROGRESS

Fig. 19




CLINICAL PROGRESS

Fig. 20



unnecessary despite their apparent value in the hands of Buckner et al (1978) and may have been the cause of the early fever. The cryophilic bacteraemia was avoidable but he produced $8 \times 10^9$ polymorphs/l in response to this 46 days after grafting.

2) Patient JW

J.W. presented with one month of epistaxis and tiredness. Despite her presentation data indicating a good prognosis she deteriorated over 5 months with progressive neutropenia, oral and vulval ulceration, skin petechiae and subconjunctival haemorrhages.

Since by referral she had received blood products from 31 donors she was regarded as highly sensitised. Anti-lymphocyte globulin (ALG) with 6 days of cyclophosphamide preconditioning were given before she received bone marrow from her HLA, MLC compatible sister. Her haematological recovery is shown in figure 21 . A chimeric state was never positively proved as the only red cell genotyping difference was the presence of S- in the donor. However, she developed biopsy proven GVHD.

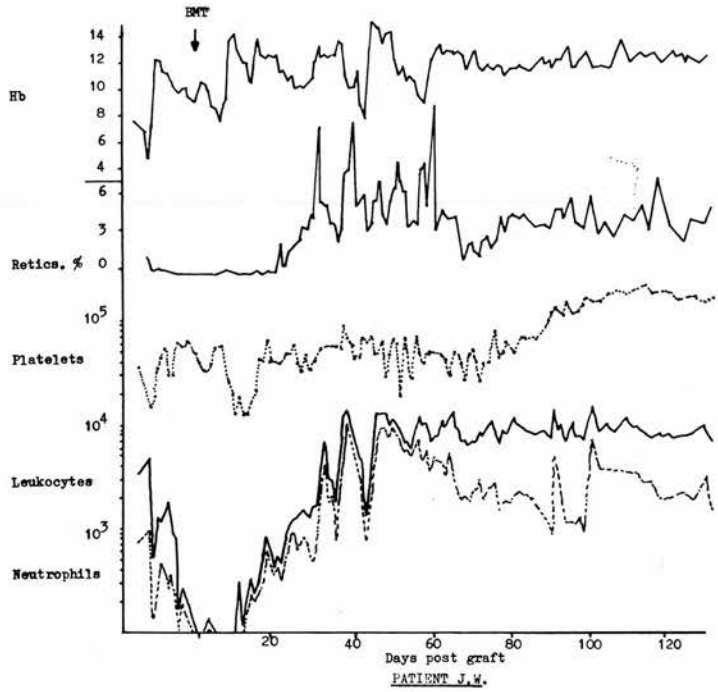
Her clinical course was stormy (Figure 22 ). A sternal rash developed immediately after the transplant without specific evidence of fat embolism. A further generalised maculopapular rash developed, sparing the palms and soles, associated with abdominal distension, a high fever and confusion but without positive evidence of bacterial or viral infection. Skin biopsy showed grade 2 GVHD with ballooning of the cells at the dermal/epidermal junction, selective cell pyknosis and separation of the epidermis from the dermis (fig. 23 ). Additional ALG and methotrexate was given. Although the fever may have already been responding all other features diminished. On day 16 when fever, confusion and the rash recurred, ALG was repeated with immediate response. Since ALG is given with 40 $mg/m^2$ of methyl-prednisolone the cause of the response may be questioned.

About day 31 she developed prolonged diarrhoea with elevated liver enzymes and serum amylase, with 10 days later more fever, confusion and two grand mal convulsions. This constellation of signs and symptoms did not respond to ALG and was ultimately shown to be due to cytomegalovirus (CMV) which was isolated from only her leucocytes. This cytomegalovirus infection

PATIENT J.W.

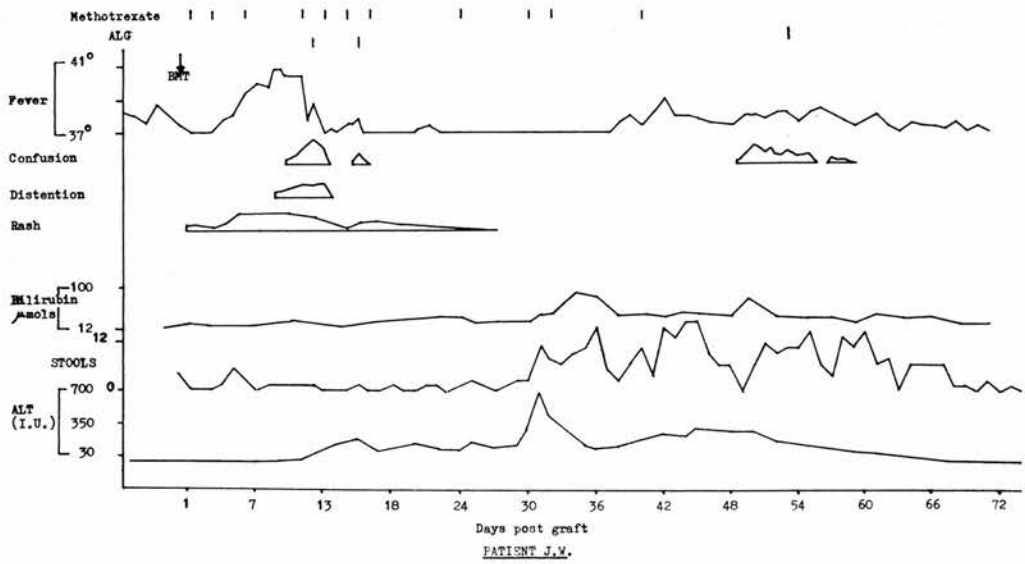
HAEMATOLOGICAL PROGRESS

Fig. 21



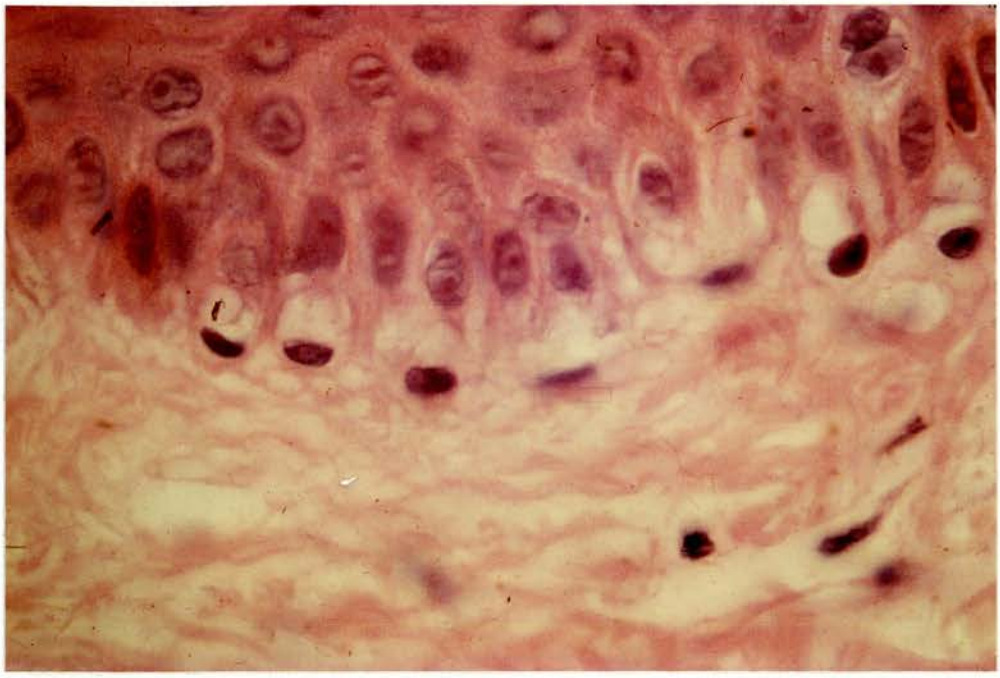

CLINICAL PROGRESS

Fig. 22

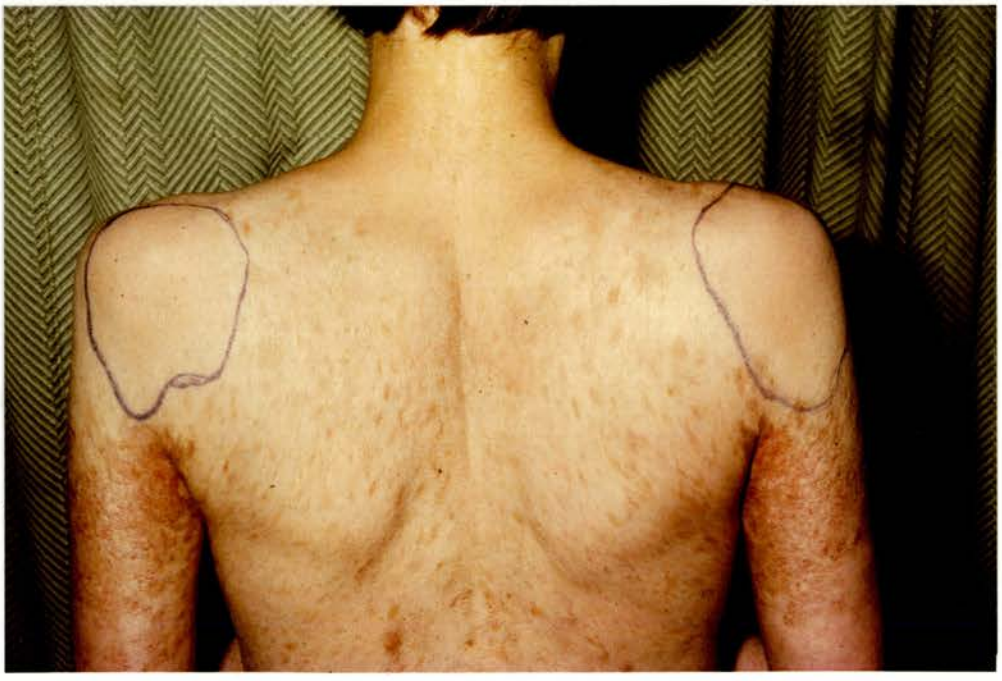


PATIENT J.W.

BIOPSY OF ACUTE GRAFT-VERSUS-HOST DISEASE Fig. 23



CHRONIC GVHD OF THE SKIN Fig. 24
(showing some clearing)



is further discussed from page 275 . Subsequently she had two episodes of pneumococcal pneumonia and one pneumococcal septicaemia between days 90 and 122.

Seven months after grafting she completed a five mile sponsored walk but three months later she showed the skin changes of mild chronic GVHD (fig. 24 ). Her skin was taut and stretched over her face and hands, with thickening and telangiectasia over her shoulders, back, thighs and arms. She had contractures of the fingers and bilateral shortening of the achilles tendons, sufficient to severely restrict mobility. She had no dermal calcification nor radiological evidence of gut involvement. Skin biopsy showed a marked increase in collagen with lymphocyte infiltration. Because the skin biopsy sites took a month to heal, the place of surgical achilles tenotomy was uncertain but this was successfully performed eight months later. Biopsies confirmed the clinical improvement in her skin despite no specific treatment and now, three years after her graft her skin is virtually normal and she works as a typist. The effects of chronic GVHD on her immunological reconstitution can be seen on page 245.

3) Patient KG

K.G. aged 9, had Fanconi's anaemia. She died of severe acute GVHD, 42 days after receiving marrow from her HLA, MLC compatible sister. Her pregraft transfusion requirements were two units of blood every eight weeks for the previous 18 months and she had received platelets on one occasion. Prednisolone and oxymethalone had given only undesirable effects. Her polymorph count remained around $0.5 \times 10^9/l$ and she had $20 \times 10^9$ platelets/l with repeated spontaneous petechiae. Conventional cyclophosphamide preconditioning and grafting was followed by six infusions of donor buffy coat in the first week. A standard methotrexate regimen but with folinic acid rescue was given as prophylaxis against GVHD.

Her clinical progress is shown in figure 25 . As the GVHD progressed she developed marrow failure, and considerable support measures were required. She developed profuse diarrhoea from the fifth day, presumably due to the cyclophosphamide or methotrexate as she had no other signs of GVHD until the fifteenth

day when a rash and raised liver enzymes were noted. She developed severe oral ulceration. Her rash desquamated and her fever and liver enzymes returned towards normal which gave false reassurance as on day 22 her palms and soles developed a maculopapular rash typical in distribution of GVHD and confirmed by skin biopsy. ALG, methylprednisolone and cyclosporin-A were given without effect and she died in progressive coma with hepatic and renal failure and progressive marrow aplasia. It is difficult to know the exact cause of death in acute GVHD but this sequence of clinical events is typical.

4) Patient MR

MR, age 15, has already been fully reported (Beard et al 1973; Barrett et al 1977). He had Fanconi's anaemia and was successfully transplanted from his compatible 9 year old brother using a modified PAPACY (Procarbazine, ALG, and cyclophosphamide) regimen. At that time there was one previous report of a successful graft in Fanconi's anaemia (Storb et al 1974). His major intercurrent problem was nutritional as he had severe dysphagia due to a lesser curve stomach ulcer. Severe disseminating ophthalmic zoster at 130 days and orbital cellulitis arising from a staphylococcal maxillary sinusitis at 145 days (fig. 26) complicated his post graft convalescence. Moderate cystitis presenting as frequency and strangury after 8 months required a number of bladder dilatations over the next two years before it resolved. He remains well and working as a painter four years after his graft.

Unsuccessful grafts

One unsuccessful aplastic graft provides an example of the difficulties encountered.

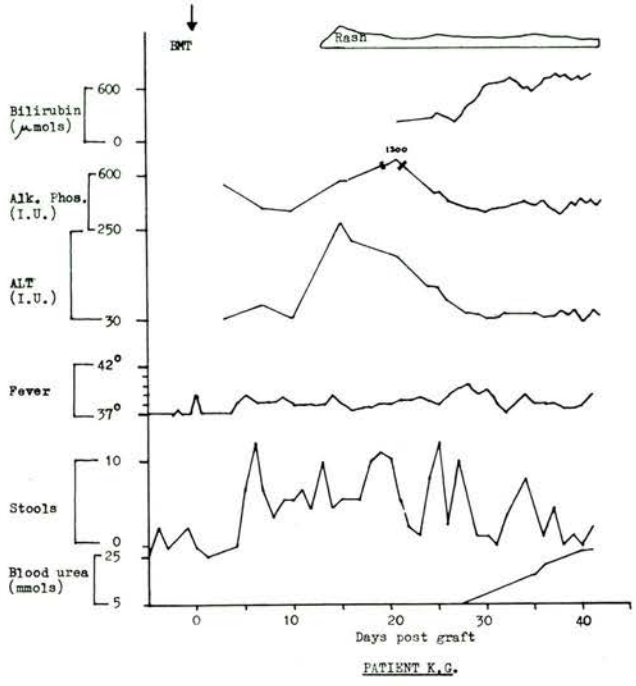
MB presented following a domiciliary psychiatric visit requested on account of severe depression. She had a haemoglobin of 1.5 gms/dl, platelets of $10 \times 10^9$/l and $0.4 \times 10^9$ polymorphs/l. Over 6 weeks there had been no response to prednisolone, oxymethalone and lithium carbonate. By the time she was grafted from her compatible brother, she had received blood products from 35 donors.

Her clinical progress is shown in figure 27 . She was

PATIENT KG

CLINICAL PROGRESS

Fig. 25




PATIENT K.G.

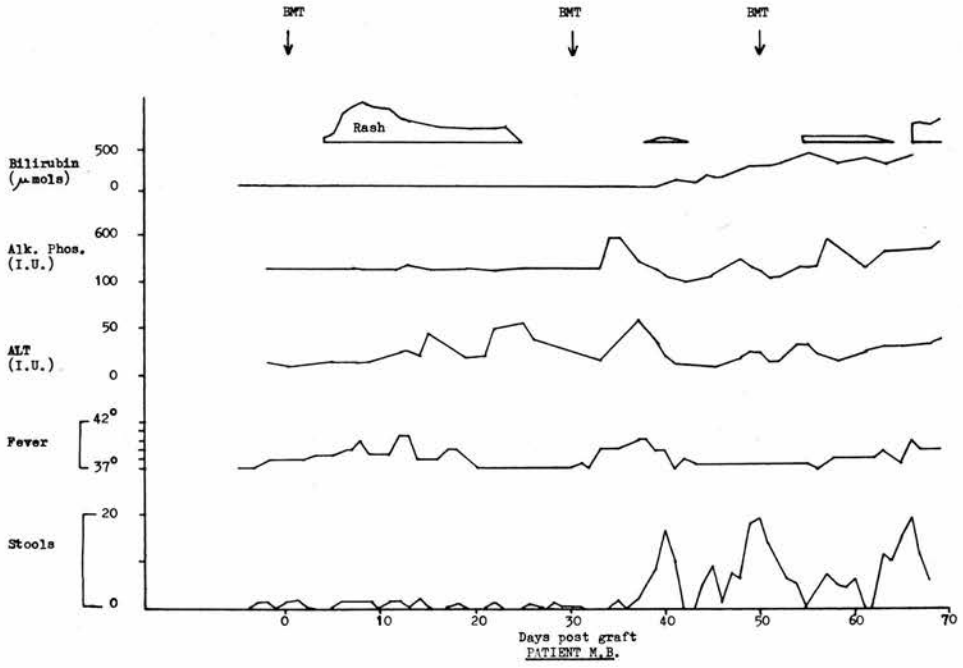
PATIENT MR

OPHTHALMIC ZOSTER WITH ORBITAL CELLULITIS

Fig. 26

UNSUCCESSFUL TRANSPLANT
FOR SEVERE APLASIA

Patient M.B. Fig. 27



febrile before the first transplant and apart from the 10 days when she received ALG and methyl prednisolone, this state persisted. There was no evidence of engraftment. She had a pseudomonas bacteraemia at day 12 and further bacteraemias 20 and 46 days later. These were related to prolonged severe neutropenia and to the start of severe diarrhoea. She also sero-converted to CMV, and excreted polyoma virus. Since the transplant had been unsuccessful, efforts were made to discern whether she had rejected the graft and whether the donor was still compatible. However her haematological indices were so depressed there were insufficient lymphocytes available to repeat the mixed lymphocyte cultures. Occasional rashes appeared, liver function deteriorated, intravenous feeding was required, blood product requirements escalated and severe diarrhoea continued with spreading perianal infection.

This patient's course shows the difficulties of transplantation in aplastic anaemia when the first graft does not lead to reconstitution. Continued neutropenia leads to infection which is unresponsive to antibiotics. Nutrition becomes difficult to maintain. Leukaphoreses and platelet support become increasingly required and less effective. HLA matched support becomes necessary and in the absence of any expectation of spontaneous recovery, team morale rapidly declines. Further less cellular transplants with risk and disruption to the donor may be attempted, but if the first graft fails, the chances of a successful second allogeneic graft in aplastic anaemia are slim (Storb et al 1978). To embark on this degree of continuous support and grafting in hope rather than expectation is not a desirable policy for a transplant team or the family involved.

## RECONSTITUTION AND SUPPORT REQUIRED AFTER GRAFTING

### Haemopoietic reconstitution

Table 85 shows the haemopoietic reconstitution of the successful transplants. The mean time from grafting to $1 \times 10^9$ polymorphs/l was 34 days and the mean time until a sustained count of $40 \times 10^9$ platelets was 27 days. For children grafted because of AML at the Royal Marsden Hospital using cyclosporin-A and not methotrexate as GVHD prophylaxis the corresponding

Table 85

HAEMATOLOGICAL RECONSTITUTION AFTER GRAFTING

| Patient | Days after grafting to | | | reticulocytes |
|---|---|---|---|---|
| | polymorphs | platelets | | |
| | $1 \times 10^9/l$ | $40 \times 10^9/l$ | $100 \times 10^9/l$ | 2% |
| MR | 30 | 34 | 50 | 29 |
| JW | 30 | 18 | 90 | 30 |
| AU | 42 | 29 | 33 | 30 |
| Aplasia (mean) | 34 | 27 | 58 | 30 |
| AML (mean) | 23 | 28 | ND | ND |

Table 86

BLOOD AND PLATELET SUPPORT REQUIRED

| Patient | No. of days to discharge or death | Units of Blood | Units of blood per day | Platelet packs | Platelet packs per day |
|---|---|---|---|---|---|
| MR | 43 | 6 | 0.14 | 14 | 0.33 |
| JW | 85 | 11 | 0.13 | 4 | 0.05 |
| AU | 65 | 2 | 0.03 | 3 | 0.05 |
| KG | 42 | 3 | 0.07 | 12 | 0.29 |
| Aplasia with graft take (mean) | 59 | 5.5 | 0.1 | 8.3 | 0.19 |
| Aplasia without graft take (mean) | 168 | 25 | 0.15 | 62 | 0.37 |
| AML grafted (mean) | 42 | 3 | 0.07 | 4 | 0.10 |

figures were 23 days and 28 days respectively. Both methotrexate and the relative 'space' created in the marrow by cyclophosphamide as compared to total body irradiation must contribute to this difference.

Table 86 indicates the blood products which may be required in the care of aplastic children undergoing marrow grafting. The amount of support per day required for those with unsuccessful transplants is considerable. Compared to those who engrafted promptly, the unsuccessful transplant recipients required 50% more units of blood and twice as many platelet packs per day. In addition they were in hospital nearly three times as long. The number of white cell donations were not comparable as these were given prophylactically to some children. The eleven most recent children transplanted for AML required half the quantity of blood and half the number of platelet packs per day as even the successful aplastic grafts. This serves to illustrate that even successful grafting for aplasia is a more intensive procedure than grafting for AML as the procedures for each now stand.

Immunological recovery after grafting

Aside from the question of immunological imbalance being the cause of acute GVHD, the immunological recovery after a transplant has two major facets. Is the patient prone to infections and do they have chronic GVHD? Immunological incompetence certainly follows acute GVHD (Witherspoon et al 1978) and chronic GVHD is associated with depression of both cell mediated and humoral immunity. In most patients these immunological deficits will be manifest as infections and table 87 shows the microbiologically proven infections suffered by three children successfully grafted for aplastic anaemia or Fanconi's anaemia. No proven infection occurred later than 145 days after grafting and all patients recovered.

Table 87

INFECTIONS AFTER SUCCESSFUL GRAFTING

(Aplasia and Fanconi's anaemia)

| Patient | Clinical presentation | Organism | Onset post graft |
|---|---|---|---|
| MR | disseminating shingles | herpes zoster | 124 days |
| | orbital cellulitis | staph. aureus | 145 days |
| AU | bacteraemia | kliebsiella sp. | 42 days |
| JW | see page | cytomegalovirus | 59 days |
| | pneumonia x 2 | strep. pneumoniae | 94 days |
| | | | 104 days |
| | bacteraemia | strep. pneumoniae | 122 days |

These patients had serial studies of their immune reconstitution performed.

Lymphocyte sub-populations (table 88 )

There is a steady increase in the number of T lymphocytes during the first 24 weeks by which time almost normal levels were achieved. Thereafter the numbers fluctuated. The high number in J.W. were associated with chronic GVHD between weeks 38 and 78, and CMV infection. Her B lymphocyte numbers show a similar rising pattern. The other two patients had normal numbers of B lymphocytes by the twelfth week after grafting. J.W. had normal numbers of $T_\mu$ and $T_\gamma$ lymphocytes, (supposedly "helper" and "suppressor" lymphocytes) at 65 weeks. In chronic GVHD, the suppressor ($T_\gamma$) lymphocytes are said to be elevated (Reinherz et al 1979) and though she did not show this by the surface marker technique used, her lymphocytes suppressed normal lymphocyte transformation to pokeweed mitogen by 96%, indicating a profound functional suppressor activity.

Table 89 shows the effect of different concentrations of thymosin fraction 5 on the peripheral blood lymphocytes of M.R. 60 days after his transplant. There is an increase to normal in the number of rosetting cells with increasing concentrations of thymosin. Further in-vitro lymphocyte studies using thymosin fraction 5 were performed to identify pre-T lymphocytes. In M.R.,

Table 88

LYMPHOCYTE SUB-POPULATIONS

(Aplasia and Fanconi transplants)

| Weeks post graft | T lymphocytes $\times 10^9/1$ | | | B lymphocytes $\times 10^9/1$ | | |
|---|---|---|---|---|---|---|
| | MR | AU | JW | MR | AU | JW |
| pre-graft | 1.1 | 0.58 | 0.72 | 0.18 | 0.12 | 0.21 |
| 4 | 0.04 | 0.09 | 0.28 | | 0.04 | 0.09 |
| 6 | 0.11 | 0.17 | | 0.08 | NE | |
| 9 | 0.31 | 0.23 | 1.16 | NE | 0.15 | 0.30 |
| 12 | 0.43 | | 1.53 | 0.15 | | 0.09 |
| 18 | 0.60 | 0.22 | 2.24 | 0.38 | 0.20 | 0.03 |
| 24 | 0.14 | 0.32 | 1.70 | 0.54 | 0.10 | 0.18 |
| 38 | 0.30 | 0.60 | 3.40 | 0,74 | 0.45 | 3.30 |
| 45 | 0.53 | 1.16 | 2.80 | 0.50 | 0.35 | 2.00 |
| 65 | 0.85 | | 1.90 | 0.72 | | 1.30 |
| 92 | | | 1.10 | | | 0.90 |
| 110 | | | 0.33 | | | 1.20 |
| Normal range | 0.47-1.8 $\times 10^9/1$ | | | 0.1-1.0 $\times 10^9/1$ | | |

NE - not evaluable

Table 89

M.R. 60 DAYS AFTER GRAFTING

| Thymosin 5 concentration/ml | T lymphocytes $\times 10^9/1$ | T lymphocytes as % of lymphocyte count |
|---|---|---|
| 0 | 0.216 | 28% |
| 0.01 µg/ml | 0.353 | 46% |
| 33 µg/ml | 0.399 | 52% |
| 333 µg/ml | 0.453 | 59% |
| 1 mg/ml | 0.607 | 79% |

the maximum increment induced with thymosin of T lymphocytes on day 60 accounts for the number of previously null lymphocytes (Table 90 ). No such increment can be demonstrated on day 130 (3 days before he developed shingles) suggesting that a maximum recruitment had occurred and the increment on day 210 is normal (Byrom 1978). AU, untroubled by infection, showed a normal increment on day + 70. A majority of J W's null lymphocytes could have the surface characteristics of T cells induced despite having considerable numbers of T lymphocytes. This reflects the considerable immune dysregulation present in chronic GVHD especially with coincident CMV infection. None of the patients had more than a small number of monocytes, the presence of which would render the separation of lymphocytes from total leucocytes much more difficult.

A marked deficit in T lymphocytes accompanied by an excess of pre-T lymphocytes might constitute an indication for thymosin therapy but there is no evidence that giving thymosin or fetal thymus transplants after allogeneic transplantation hastens the reconstitution of immunity or prevents GVHD or the immune dysregulation associated with GVHD (Atkinson 1980).

T lymphocyte function

Figure 28 shows the increments obtained in-vitro with lymphocytes from M.R. in response to PHA, allogeneic cells and candida immunogen. Responses before the graft were extremely low and progressive deficiency of cell mediated immunity has been reported in Fanconi's anaemia (Pedersen et al 1977). After the graft there was a marked increase in response to PHA and allogeneic cells and finally to candida immunogen. The responses at day 150 were diminished probably due to recent varicella/ zoster and staphylococcal orbital cellulitis. In-vitro candida killing at that time was reduced but staphylococcal killing was normal.

A.U. (fig. 29 ) showed normal in-vitro T lymphocyte function by day 41 after grafting but cutaneous delayed hypersensitivity to candida was not detected until 3 months later, probably reflecting local monocyte function or T lymphocyte/ monocyte interaction. No circulating inhibitors of T lymphocyte responses to candida were identified.

Table 90

## EFFECT OF THYMOSIN IN INDUCTION OF T LYMPHOCYTES

| Patient | days post graft | lymphocytes x $10^9$/l | | | | max post thymosin | increment induced |
|---|---|---|---|---|---|---|---|
| | | total | B | T | null | | |
| MR | +60 | 0.77 | 0.15 | 0.22 | 0.40 | 0.61 | 0.39 |
| | +130 | 1.06 | 0.38 | 0.60 | 0.09 | 0.60 | 0 |
| | +210 | 0.75 | 0.14 | 0.27 | 0.34 | 0.33 | 0.60 |
| AU | -20 | 1.77 | 0.12 | 0.58 | 1.06 | 0.78 | 0.20 |
| | +70 | 0.56 | 0.15 | 0.22 | 0.19 | 0.25 | 0.03 |
| JW | +106 | 5.33 | 0.04 | 2.24 | 3.05 | 5.00 | 2.76 |
| | +171 | 7.38 | 0.18 | 1.70 | 5.50 | 3.94 | 2.24 |

Normal increment 0-0.4

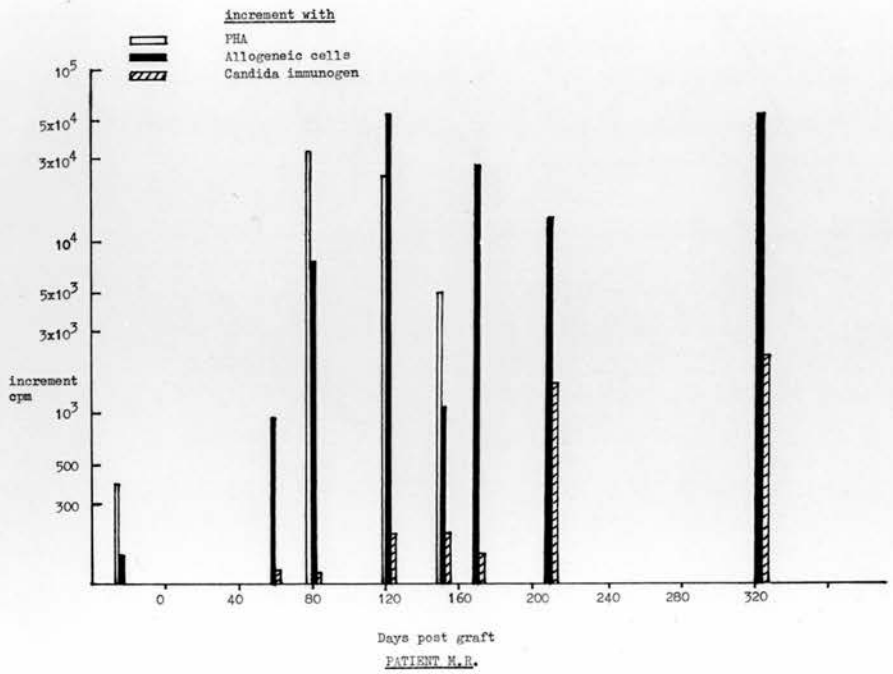
Normal range T lymphocytes 0.47-1.8 x $10^9$/l

B lymphocytes 0.1-1.0 x $10^9$/l

(Byrom 1978)

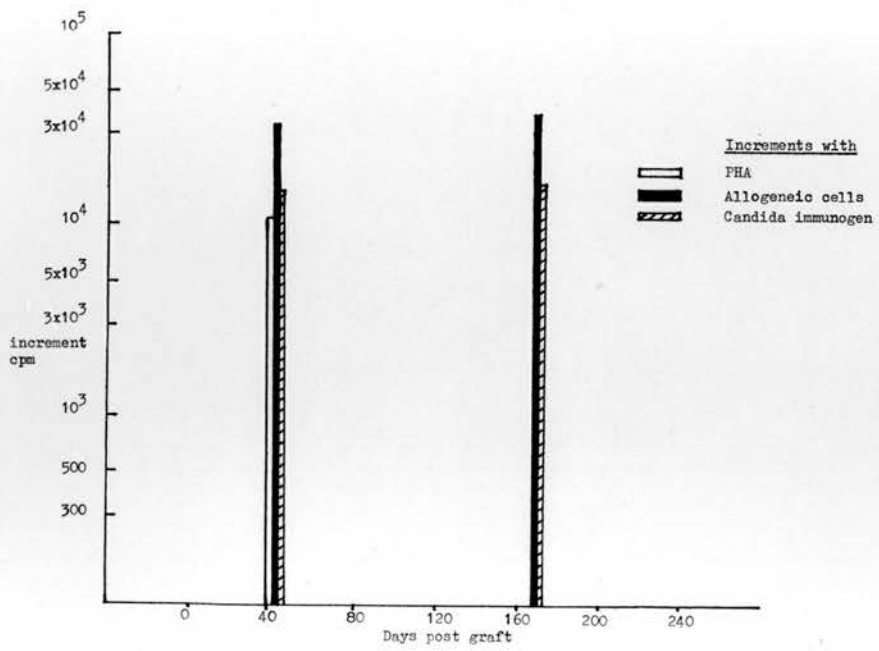
# IN-VITRO T LYMPHOCYTE FUNCTION

## patient M.R. (fig.28 )




PATIENT M.R.

## patient A.U. (fig. 29 )




PATIENT A.U.

J.W. showed good recovery of in-vitro T lymphocyte function by day 130 (fig. 30 ) with subsequent depression during chronic GVHD with CMV. There was also a massive elevation of immunoglobulin G levels. T lymphocyte responses at this time were increased up to thirty fold in normal plasma compared to her autologous plasma indicating that her plasma caused considerable inhibition of these responses.

Immunoglobulin levels (table 91 )

M.R. had two transient depressions of immunoglobulin (Ig) G, one in the first month after his graft and one at three months. The levels were virtually normal at 18 weeks before he suffered disseminating zoster and orbital cellulitis. A.U. showed no real abnormality of his immunoglobulin levels at any time following his transplant. J.W. showed a massive increase in IgG associated with very high CMV antibody levels and chronic GVHD. This was a normal polyclonal antibody and no free chains were present in the urine. None of these children showed any significant abnormality of IgA or IgM. The lowest isohaemagglutinin titre was in J.W. at week 37 with an anti-B titre of 1/8, virtually the same as her pregraft titre of 1/16. A.U. showed a three tube depression of his pre-graft anti-B titre during week 6 but this recovered by week 24. All complement ($C_3$, $C_4$) studies were normal.

Discussion

These three patients had almost normal T and B lymphocyte numbers by 12 weeks after their grafts. There was good evidence of thymosin induction of T lymphocytes in all patients and of suppressor activity in the one patient with chronic GVHD. In-vitro responses to PHA were normal by 11 weeks in two but not until one year in one patient. Responses to allogeneic cells were normal in all three by 18 weeks and responses to candida immunogen normal by 18 weeks in two patients and one at one year. Immunoglobulin levels were normal in two by 8 weeks and in one by 18 weeks. These three patients had all but normal immunological studies by 18 weeks (126 days) after grafting although the patient who developed chronic GVHD showed subsequent severe immune disturbance at that time. Two suffered intercurrent

# IN-VITRO T LYMPHOCYTE FUNCTION

patient J.W. (fig. 30 )

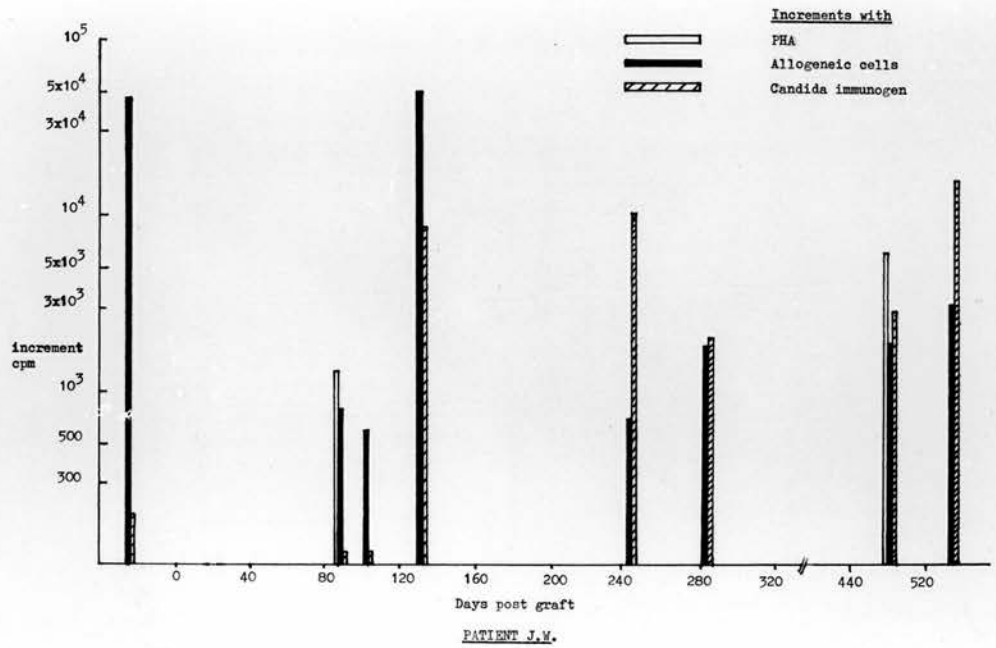


Table 91

IMMUNOGLOBULINS (gms/l) AFTER GRAFTING

(Aplasia and Fanconi's anaemia)

| Patient | MR | | | AU | | | JW | | |
|---|---|---|---|---|---|---|---|---|---|
| weeks post graft | IgG | IgA | IgM | IgG | IgA | IgM | IgG | IgA | IgM |
| pre-graft | 9.4 | 0.8 | 0.25 | 7 | 0.7 | 1.1 | 5.8 | 0.8 | 0.9 |
| 0 | 8.6 | 1.0 | 0.4 | | | | | | |
| 2 | 3 | 0.4 | 0.4 | | | | | | |
| 4 | 4.3 | 0.5 | 0.3 | | | | 7 | 0.5 | 0.4 |
| 8 | 5.8 | 0.4 | 0.2 | 8.2 | 0.5 | 0.7 | 3.7 | 0.3 | 0.4 |
| 14 | 2.6 | 0.25 | 0.3 | | | | | | |
| 18 | 7 | 0.6 | 0.3 | 6.0 | 0.3 | 0.4 | 13.6 | 0.1 | 0.4 |
| 28 | 16 | 1.4 | 1.1 | | | | 26 | 0.6 | 1.0 |
| 38 | | | | 7.2 | 0.3 | 0.6 | 45 | 0.5 | 1.0 |
| 45 | | | | | | | 45 | 0.5 | 1.0 |
| 67 | | | | 6.2 | 0.4 | 0.8 | 33 | 0.5 | 1.4 |
| 77 | 10.4 | 2.3 | 0.8 | | | | 28 | 0.4 | 1.6 |
| 110 | | | | | | | 24 | 0.4 | 1.3 |
| 150 | | | | | | | 15.2 | 0.5 | 1.4 |

Normal range IgG 5–14 gms/l

IgA 0.5–2.6 gms/l

IgM 0.5–2.0 gms/l

severe infections but none later than 145 days after grafting.

Gale et al (1978) found in a mixed group of grafted aplastic and leukaemia patients that T and B lymphocyte numbers were normal by 7 weeks as was the response to allogeneic cells. PHA responsiveness was depressed in one-third until one year. No particular depression of immunoglobulins was found. Elfenbein et al (1976) reported a very mixed group of transplant patients in Baltimore with similar results but found isohaemagglutinin levels depressed for a year and great variation in the return of delayed hypersensitivity. The Seattle data (Storb et al 1976) suggested that T and B lymphocyte numbers, immunoglobulins G and M, and complement levels were restored by three months, in-vitro T lymphocyte function by five months, but immunoglobulin A levels, antibody responses to $\phi$x 174, isohaemagglutinins and cutaneous delayed hypersensitivity tests took more than one year to return to normal. No particular difference in the return of immunity after successful grafting has been associated with different preconditioning regimens, and methotrexate post-graft regimens probably have little intrinsic effect. Witherspoon et al (1978) showed that GVHD had considerable effect on immune reconstitution and was associated with impairment of immunity even if no longer present. Fatal infections are most unusual in the absence of GVHD in British patients who have returned home after bone marrow grafting. The vast majority of patients without GVHD who survive four months after a marrow transplant (no matter the primary diagnosis) have no real problem with infection.

# MARROW TRANSPLANTATION OF ELEVEN CHILDREN WITH ACUTE MYELOID LEUKAEMIA

## Introduction

Chemotherapy with or without cranial irradiation is the initial treatment for acute leukaemia. Induction of remission is followed by maintenance of remission which may or may not include episodes of pseudo-reinduction, intensive chemotherapy or immunotherapy. The results of this conventional therapy determine the place of such alternative measures as allogeneic marrow grafting.

Acute lymphoblastic leukaemia (ALL) in childhood of the common type (c ALL) will almost always remit with chemotherapy and this initial remission is maintained in about 45% of patients until treatment is stopped between two and three years later (George et al 1979). About 55% of children with cALL therefore will have recurrent disease and of these very few become long term survivors, the median survival being 8-9 months after first relapse. (Kung et al 1978). T-cell leukaemia and more especially those who present with a high white cell count have a worse prognosis from the outset resulting in about 5-10% of males and perhaps 25% of females being long-term survivors following conventional therapy. Conventional treatment of B cell leukaemia is highly ineffective and the results of treating null cell leukaemia, though poor, are more variable as a proportion with null cell disease probably have one of the other forms without exhibiting the cell surface characteristics. Conventional therapy appears to cure a proportion of children with ALL but a substantial number will need an alternative therapy if a cure is sought.

Chemotherapy is much less effective in curing acute myeloid leukaemia (AML). Most series report 5-15% long term survivors but the median survival of the 70% who obtain a first remission is 16 months (Powles et al 1980). Once relapse occurs the median survival is three months. Although these data relate predominantly to adults, there is little evidence that childhood AML behaves in any different manner, although recent results with chemotherapy and immunotherapy may be a little more encouraging (MRC AML 1981).

The evidence very much suggests that a majority of children with AML in remission, high count cALL, relapsed common ALL and possibly T cell ALL will die within eighteen months. Despite the dangers of marrow transplantation it is reasonable to consider these children as candidates for matched allogeneic marrow grafting if a cure is being sought. The state of the art is not yet such that incompletely compatible grafts can be easily recommended although this is a coming option.

The results of marrow transplantation

The early results of bone marrow transplantation in acute leukaemia are reflected in the variety of preconditioning regimens used at different centres (table 92 ), all with poor survival rates. Of 206 patients in these series, 21 were 'actuarial' survivors, a rate of 10% overall. In general the regimens with the least recurrent leukaemia (SCARI; CY-TBI-BCNU) had many early deaths from complications of the preconditioning regimen, or GVHD. Thomas et al (1977) reported 100 transplant recipients with a minimum follow-up of one year. Seventeen were alive, three of whom had chronic GVHD and four who had recurrent leukaemia. Gale (1979) from 1969-1975 had similar results with 18% survivors more than two years after grafting compared with no survivors in his coincident chemotherapy series. Many patients in these series had leukaemia resistant to conventional therapy so results of 17-18% long term survivors were remarkable in that any long term survival was achieved.

The massive cytoreductive therapy (table 92 ) gave major complications and yet still the leukaemia returned in many patients. Patients in remission who had already achieved a 2-3 log decrease in the amount of leukaemic tissue might show better results and interest turned to performing elective transplants in remission. Buckner (1979) reported 89 patients with acute leukaemia transplanted in 1976 and 1977. Those transplanted in relapse had a one year survival of 18% (a figure similar to their unit's earlier results quoted by Thomas et al 1977) whereas those transplanted in remission (less than 5% leukaemic blasts in the marrow and a normal CSF) had a one year survival of 55%. All patients received the same preconditioning, cyclophosphamide and TBI, plus marrow from

Table 92

SURVIVAL RATES WITH DIFFERENT PRECONDITIONING REGIMENS USED IN MARROW GRAFTING FOR ACUTE LEUKAEMIA

| Regimen | No. of patients | Survivors | (%) |
|---|---|---|---|
| TBI | 10 | 1 | 10 |
| CY | 34 | 0 | 0 |
| CY-TBI | 43 | 6 | 14 |
| CY-TBI-DNR | 29 | 3 | 10 |
| CY-TBI-BCNU | 18 | 3 | 17 |
| CY-TBI-Ara-C | 9 | 2 | 22 |
| BACT | 10 | 0 | 0 |
| SCARI | 30 | 4 | 13 |
| DAFT | 23 | 2 | 11 |

(Gale 1979)

TBI - total body irradiation

CY - cyclophosphamide

DNR - daunorubicin

BCNU- carmustine

BACT ) acronyms describing regimens involving irradiation,
SCARI ) anthracyclines, nitrogen mustards, cyclophosphamide,
DAFT ) cytosine arabinoside and 6-thioguanine

a matched sibling.

This same advantage is shown in the results of Thomas et al (1979) who reported patients with ALL transplanted at least 15 months previously. Of 22 patients transplanted in second or subsequent remission, eleven were alive compared with only four of 26 transplanted in relapse. In AML, Thomas et al (1979) reported that twelve of 19 patients transplanted in first remission were alive at least one year later and a mean of more than 17 months after achieving complete remission. Powles et al (1980) properly and cautiously describing the follow-up after grafting as 'continued duration of first remission' reported that 18 of 22 patients grafted because of AML continued in remission compared to nine of 28 who had not received a transplant. The median duration of remission of their patients receiving conventional maintenance therapy was 52 weeks and that of the transplanted patients had not been reached but was in excess of 45 weeks.

Grafting in remission gives better survival than grafting in relapse and it seems likely from the results of Thomas et al (1979) and further follow-up of the patients of Powles et al (1980) that grafting in AML gives both longer survival and longer duration of first remission than conventional therapy. With ALL in second or subsequent remission, the results quoted are encouraging compared with conventional therapy but longer follow-up is required to assess the ultimate benefit. Disease-free survival is certainly prolonged.

## The additional dangers of marrow grafting

### Infection

There are dangers intrinsic to marrow grafting over and above those in conventional therapy. Infection during the period of severe neutropenia and during immune reconstitution, GVHD, interstitial pneumonitis and a presumed risk of second tumours have to be added to the chance of recurrent leukaemia. The risk of dying from infection during the transplant procedure or the succeeding three months is small although fevers and systemic antibiotic therapy are common as are septicaemia and major local infections. The incidence of these problems

is considerably reduced by the use of a protected environment and prophylactic antimicrobial agents (Buckner et al 1978) although these protective measures made no difference to the incidence of GVHD, interstitial pneumonitis or ultimate survival. Of 89 leukaemia patients transplanted in Seattle, four died of pyogenic infection shortly after grafting (Buckner 1979) as did two of thirty-three in Duarte, California (Blume et al 1980); three (of fungaemia) of fourteen in Los Angeles (Gale et al 1976) and none of twenty-two (Powles et al 1980) in London. The high figure from Los Angeles reflects the very toxic programme then being used.

Interstitial pneumonitis

Interstitial pneumonitis is associated with infection, GVHD, radiation and the allogeneic graft but the precise association between these factors remains difficult. In addition some leukaemia remission induction regimens contain drugs known to be toxic to the lung parenchyma and methotrexate is often given after grafting as prophylaxis against GVHD. Centres in the USA have a greater incidence of interstitial pneumonia than centres in Europe, but whether this reflects genetic, environmental or therapeutic differences is unclear. The median onset is about 52 days after grafting with a few days of fever and cough followed by progressive dyspnoea, hypoxia and relatively unimpressive chest signs compared with the diffuse infiltrate on chest x-ray. The aetiology and outcome of the interstitial pneumonitis seen in 89 leukaemia transplant patients in Seattle are shown in table 93 (Buckner 1979). 60% of patients died, the highest death rate being associated with CMV infection.

In the United Kingdom, Powles et al (1980) reported one fatal case out of 22 AML transplant patients, but on the West Coast of the USA the incidence is 40-50% of patients transplanted for acute leukaemia and 10-30% of patients transplanted for acute aplasia. Although aplastics did not receive TBI, many received additional immunosuppression in the form of ALG, given on account of GVHD. If GVHD was sufficient to require ALG, the incidence of interstitial pneumonitis rose from 27% to 78% of patients (Neiman et al 1977), and patients transplanted in relapse are more likely to suffer interstitial pneumonitis

Table 93

AETIOLOGY AND OUTCOME OF INTERSTITIAL PNEUMONITIS IN 89 TRANSPLANTS FOR ACUTE LEUKAEMIA (Buckner 1979)

| Organism | No. of cases | No. of deaths | % died |
|---|---|---|---|
| Cytomegalovirus | 12 (34%) | 10 | 83 |
| Pneumocystis | 5 | 2 | 40 |
| Herpes simplex | 1 | 1 | 100 |
| Idiopathic | 17 (50%) | 8 | 47 |
| Total | 35 | 21 | 60 |

than those grafted in remission (50% compared to 32%) (Buckner 1979). Leukaemic patients receiving syngeneic grafts had an incidence of 8% (Neiman et al 1976) so postgraft methotrexate or the actual allogeneic graft must play a part. The latter is perhaps supported by the 50% incidence of overwhelming pulmonary oedema following incompatible bone marrow transplants for AML seen at the Royal Marsden Hospital when cyclosporin-A not methotrexate was used as GVHD prophylaxis (Powles 1981). There are no references to an aplastic patient receiving syngeneic marrow who developed interstitial pneumonitis.

Graft-versus-host disease

In an earlier series of 100 transplants (Thomas et al 1977), acute GVHD affected 80% of patients, was moderate or severe in 64% of these and was implicated in the death of 37% of the transplanted patients. The fatalities due to acute GVHD increased if donors were less than fully HLA, MLC compatible but a donor of the opposite sex to the patient did not increase the risk. (Bortin and Rimm 1978). The incidence of GVHD was unaffected by whether the patient was in relapse or remission at the time of grafting but the incidence of recurrence of leukaemia was reduced in those surviving moderate to severe GVHD. This was no real benefit as the number dying as a consequence of GVHD compensated for any gain achieved. Syngeneic grafts avoid the GVHD mortality but have a higher incidence of recurrent leukaemia and syngeneic recipients were at an overall advantage, with around 30-35% long term survivors (similar to those with grade I GVHD) compared to the overall 18% survival of those transplanted in relapse, but there may have been some patient selection.

The death rate from acute GVHD in first remission AML patients has fallen to about 8% recently (Atkinson 1980). This was coincident with Powles et al (1980) reporting the prophylactic use of cyclosporin-A until at least three months after grafting, a policy resulting in an acute GVHD death rate of 5% (compared to 20% previously). Seattle does not as yet use cyclosporin-A so an open question remains on the specific value of cyclosporin-A in the matched allogeneic sibling graft performed early in first remission of AML. Controlled trials (versus methotrexate)

should resolve at least part of this question.

Chronic GVHD remains a problem. Table 94 shows that in recent publications 13 of 73 survivors (17%) have chronic GVHD and an incidence of 33% may prevail. This has been usually mild or confined to one system only. Powles et al (1980) reported the lowest incidence but the shortest follow-up and their median follow-up after stopping prophylactic cyclosporin-A was 5 weeks. Fifteen percent of their reported patients who have been observed for more than a year after their transplant have now developed chronic GVHD.

Recurrent leukaemia and tumours

This is not really an additional risk specific to grafting but is an important sequela. It is almost always of the original type and only eight reports exist of leukaemia being proved to be of the donor karyotype. Followed up for between 15-35 months (Thomas et al 1979) 10 patients relapsed of 22 ALL patients transplanted in second or subsequent remission compared with 15 relapses in 20 patients transplanted in relapse. The chance is therefore considerable. A history of, or active CNS disease, doubled the likelihood of recurrent leukaemia. While this incidence of recurrent leukaemia is not encouraging there is no doubt that the projected survival of 50% at 2 years after grafting is better than would be expected from the natural history of ALL in second or subsequent remission.

The group with ALL transplanted in second or subsequent remission included 13 children below the age of 10 of whom 7 are alive without leukaemia, a mean of over 2 years later. Three of these seven were in their third or fourth remission and two had active CNS disease at the time of grafting. A further two children are alive but in relapse. The latest time of relapse was 55 weeks post graft for those transplanted in relapse and 40 weeks for those transplanted in remission. Remembering that chemotherapy nearly always fails to maintain a second remission, transplantation would seem to have a valuable role in half the children, and the difference between the results for children and adults is becoming increasingly apparent, both in transplantation for ALL and AML.

Table 94

INCIDENCE OF CHRONIC GVHD IN RECENT TRANSPLANT SURVIVORS

| Author | Disease | Patients transplanted | Survivors | No. with chronic GVHD | Follow-up |
|---|---|---|---|---|---|
| Thomas et al 1977 | AL | 100 | 17 | 3 (18%) | 1-4 years |
| Thomas et al 1979 | AML ($1^{o}$ rem) | 19 | 12 | 4 (33%) | median 18 months |
| Powles et al 1980 | AML ($1^{o}$ rem) | 21 | 16 | 1 (6%) | median 7 months |
| Thomas et al 1979 | ALL rem | 22 | 11 | 3 (27%) | 15-35 months |
| Thomas et al 1979 | ALL rel | 26 | 4 | 0 (0%) | 15-35 months |
| Blume et al 1980 | AL | 33 | 13 | 2 (15%) | 8-33 months |

AL Acute leukaemia
AML Acute myeloid leukaemia
ALL Acute lymphoblastic leukaemia
rem Remission
rel Relapse

With AML transplanted in first remission Thomas et al (1979) reported one relapse in 19 patients which occurred in the 24th week after grafting. Twelve patients have survived longer than this, their mean survival after grafting being greater than 100 weeks. Powles et al (1980) report 3 relapses at a mean of 52 weeks post graft in 20 allogeneic transplants for AML, although there is doubt in one patient about the original diagnosis of AML. If that patient had ALL then his lymphoblastic relapse at 93 weeks after grafting is an ominous portent. Thirteen patients (65%) have survived disease-free for a mean of 74 weeks, including 14 weeks between complete remission and transplantation, whereas of their control group of 28 patients, 8 (28%) have survived disease-free for a mean of 61 weeks from complete remission. The reported numbers of children are too small to draw any useful conclusion but there is no evidence they behave any differently. So also in AML it would seem that transplantation has a useful role to play both as regards survival and disease-free survival.

Should the patient relapse after grafting, their marrow is of course that of the donor and so very toxic chemotherapy can be given followed by more donor marrow to curtail the period until marrow recovery - a 'pseudo-syngeneic rescue'. Whether or not there will be an increased incidence of second tumours in survivors following a transplant for acute leukaemia is impossible to say. Control data from ALL patients surviving after second remission or AML patients is too scanty and the adults transplanted and surviving when cyclosporin-A prophylaxis was used have not been followed long enough. Second tumours in aplastic patients successfully transplanted with cyclophosphamide are rare but the use of TBI in patients who have already suffered a haematological malignancy must increase the risk. However it is clear that at least during a five year follow-up in Seattle that second tumours are extremely rare.

The results of matched sibling marrow transplants are sufficiently encouraging for such marrow transplantation in remission to be indicated in AML, B cell ALL, boys with high risk ALL in first remission and in all children with ALL in second remission.

## CLINICAL MARROW TRANSPLANTATION FOR CHILDHOOD AML

In the past 18 months eleven patients, aged 16 years or less with AML have received bone marrow transplants at the Royal Marsden Hospital. All were in remission at the time of grafting, this having been achieved with either the MRC protocol or other combinations of anthracycline, cytosine arabinoside, 6-thioguanine and steroids. Three children were in second remission, the others in initial remission. All received some form of maintenance therapy between remission and grafting. One (A.B.) had extradural cord compression at presentation and three had infection of a nature and course requiring white cell support during their remission induction regimen. Table 95 shows the FAB diagnosis; the problems encountered during remission induction and the time taken to achieve a first complete remission.

Donor compatibility data and grafting details are shown in table 96 . Seven children received grafts from an HLA, MLC compatible sibling, one from a phenotypically HLA identical MLC unreactive father and three from their one haplotype identical MLC reactive father. The mean graft size was $3.44 \times 10^8$ nucleated stem cells per kg. recipient body weight. G.C. required a second graft after the first graft was suppressed by cytosine arabinoside given for suspected progressive multifocal leukoencephalopathy. The interval between achieving full remission and marrow grafting is shown and the mean interval between diagnosis and transplantation was 33 weeks.

All received the same pretransplant conditioning; cyclophosphamide 1.8 gms/$m^2$ on days minus four and minus three, with 1000 rads TBI on day minus one and cyclosporin-A 12.5 mg/kg per day also starting on day minus one as prophylaxis against GVHD. The four children who received paternal marrow also received methotrexate GVHD prophylaxis. All were nursed in a protected environment and received either NEOCON or TSN as antimicrobial prophylaxis.

Their problems after grafting while still in-patients are summarised in table 97 . Nine of the eleven children became febrile and received systemic antibiotics. Six developed skin rashes of which five were histologically proven to be GVHD, and four of these five had received partially matched transplants

Table 95

PATIENTS AGED SIXTEEN YEARS OR LESS
TRANSPLANTED FOR ACUTE MYELOID LEUKAEMIA

| Patient | Sex | Age | FAB diagnosis | Major problems during remission induction | Time from diagnosis to this remission (days) |
|---|---|---|---|---|---|
| C.C. | F | 14 | M4 | None | 48 |
| E.H. | F | 11 | M2 | Infection requiring white cell support | 31 |
| S.L. | M | 11 | sub-acute M5 | None | 116 |
| J.G. | F | 13 | M6 | Septicaemia requiring white cell support | 295 |
| G.C. | F | 6 | M4 | None | 40 |
| A.B. | M | 16 | M2 | Extradural cord compression | 44 |
| L.S-F. | F | 15 | M4 | None | 46 |
| T.T. | F | 14 | M6 | None | 186 |
| A.F. | M | 14 | M2 | Pneumonia | 60 |
| L.F. | M | 14 | M4 | Infection requiring white cell support | 49 |
| S.A. | M | 14 | M2 | None | 137 |

Table 96

COMPATIBILITY AND TRANSPLANT DATA

| Patient | Donor | HLA A,B,C,D identical | MLC compatible | Size of graft nucleated cells/ kg body wt | Interval between this remission and grafting (days) |
|---|---|---|---|---|---|
| C.C. | Brother | yes | yes | 3.0 | 44 |
| E.H. | Brother | Yes | yes | 3.7 | 296 |
| S.L. | Father | A and B only | no | 3.5 | 77 |
| J.G. | Sister | yes | yes | 3.4 | 76 |
| G.C. | Father | yes | yes | 4.1 and 3.5 | 89 |
| A.B. | Sister | yes | yes | 3.1 | 75 |
| L.S-F. | Brother | yes | yes | 3.3 | 44 |
| T.T. | Father | one haplotype | no | 3.7 | 15 |
| A.F. | Father | one haplotype | no | 3.6 | 113 |
| L.F. | Brother | yes | yes | 3.3 | 530 |
| S.A. | Sister | yes | yes | 3.1 | 155 |

Table 97

PROBLEMS WHILE IN-PATIENTS

| Name | Hospital stay post graft | Nature of problem | Maximum weight loss |
|---|---|---|---|
| C.C. | 28 days | Rash ? cause<br>urinary infection (strep. faecalis) | none |
| E.H. | 21 days | Bacteraemia (staph. aureus) | 5% |
| S.L. | 43 days | Diarrhoea<br>bacteraemia (staph. albus)<br>rash - GVHD grade 2<br>psychological withdrawal<br>intravenous feeding | 15% (4.5 kg) |
| J.G. | 30 days | Convulsions<br>cytomegalovirus excrection | none |
| G.C. | 83 days | Diarrhoea<br>rash - GVHD grade 2<br>convulsions and brain damage<br>marrow suppression by cytosine<br>intravenous feeding for 60 days | 15% (3 kg) |
| A.B. | 19 days | None | none |
| L.S-F. | 24 days | None | 1.6% |
| T.T. | 26 days | Rash<br>severe mucositis<br>GVHD - grade 3 | 8% (4 kg) |
| A.F. | 80 days+ | Rash - GVHD grade 2<br>convulsions<br>severe disseminated herpes zoster<br>cellulitis of arm<br>transverse myelitis and paraplegia<br>urinary infections | 10% (8kg) |
| L.F. | 30 days | Urinary infection | 7% 4.2 kg |
| S.A. | 40 days | Rash - GVHD grade 2<br>generalised chickenpox | none |

from their father (table 96 ). One of the seven children who received a matched allogeneic graft developed GVHD. All ultimately responded to high dose methyl prednisolone. Two children had marked diarrhoea following TBI, without any qualitative microbiological difference being detected in their stools compared with those of the other patients. These two children lost more than 10% of their body weight and received intravenous and supplemental feeding. Four children had no net weight loss by discharge. Two children had proven bacteraemias and three had urinary tract infections. One excreted cytomegalovirus in association with a convulsion and two with neurological complications shed BK polyoma virus in the urine. One who developed chickenpox 4 days after his transplant was treated with acyclovir and showed no untoward effects. One boy showed marked psychological withdrawal during isolation, despite full parental access and did not return to normal until 4 weeks after discharge home.

Problems after discharge and their timing are shown in table 98. Five patients had flattening of their affect, nausea, vomiting and weight loss with tiredness and debility which improved from about the tenth week. These symptoms were due to the after-effects of a major hospital procedure and an in-patient stay in a confined cubicle for almost six weeks combined with the known side effects of cyclosporin-A and the late effects of radiation. Almost all children have shown some degree of tremor or clumsiness while taking cyclosporin-A and it is becoming increasingly possible that cyclosporin-A contributed to the serious neurological problems seen. However, blood levels of cyclosporin-A can only now be measured and the excessive levels found retrospectively in these two children will be avoided in future. Two children developed skin GVHD after discharge which responded rapidly to doubling of the dose of cyclosporin-A. Infections have been a minor problem. Two developed oral herpes simplex lesions and two suffered shingles. The patient with the longest follow-up showed some increased pigmentation especially in sites of previous trauma but this tendency seems to be most marked in those of Mediterranean descent. One patient relapsed at 6 months and is now dead.

G.C., who received a graft from her father, had a major

Table 98

PROBLEMS AFTER DISCHARGE

| Patient | follow up since graft (weeks) | week post graft | Problem | Present status |
|---|---|---|---|---|
| C.C. | 82+ | 12 | Mucocutaneous herpes simplex | very well, at school |
| | | 24 | Shingles | |
| | | 42+ | Pigmentation of sites of previous trauma | |
| E.H. | 47+ | 5 | GVHD of skin (grade 3) | very well, at school |
| | | 7 | Flattened effect | |
| S.L. | 49+ | to 11 | Psychological withdrawal | very well, at school |
| | | 20 | Nausea and vomiting due to Septrin | |
| | | | Shingles | |
| J.G. | 43+ | to 9 | Vomiting, nausea and weight loss | very well, at school |
| | | 10 | Transient lymphadenopathy | |
| | | 12 | Herpes simplex of lip | |
| G.C. | 61+ | 18 | Further convulsions | severely mentally retarded chronic GVHD |
| | | 20-27 | Progressive hypogamma-globulinaemia | |
| | | 20+ | Severe intellectual impairment | |
| A.B. | 37 (died) | 8-11 | Flattened effect | dead |
| | | 12 | Rash | |
| | | 28 | Relapse | |
| L.S-F. | 26+ | 8+ | Dizziness (intermittent) | very well, at school |
| | | 15+ | Constipation | |
| T.T. | 22+ | 8 | | very well, at school |
| | | 10+ | GVHD (grade I) | |
| A.F. | 14+ still in-patient | | See previous table | Transverse myelitis improving slowly |
| L.F. | 12+ | | None | Very well, |
| S.A. | 8+ | | None | v. mild GVHD |

immunological problem and now has chronic GVHD. Her first graft was severely compromised by cytosine arabinoside given to treat possible multifocal leukoencephalopathy. A second graft from the same donor gave prompt haematological reconstitution. However, from that time her immunoglobulin G levels declined as her lymphocyte count recovered. IgA and IgM levels remained virtually normal and no immunoglobulin abnormalities have been observed in any of the other patients transplanted for leukaemia unless they also had GVHD. She had a very high number of activated suppressor T lymphocytes, the sex of which has yet to be determined. "Her" lymphocytes suppressed the production of IgG by normal lymphocytes in response to pokeweed mitogen by 95%. Her father has had no infective problems and has normal immunoglobulin levels. However, his lymphocytes show a mildly suppressive action on normal immunoglobulin production, though he has no excess of activated suppressor T lymphocytes. In the absence of any other explanation, it would seem likely that a suppressor clone, present but under control in father, has been grafted but without whatever controlling influence exists in father. Stopping her cyclosporin-A did not alter the situation.

Six of seven alive more than twenty-two weeks after their transplant are at school and leading a normal life.

Haematological recovery and support

The mean stay in hospital for these children was 8 days before the graft and 34 days after the graft, slightly longer than the overall mean of 38.5 days quoted by Kay et al (1980). None required white cell transfusions (table 99 ) but on average each child required 3 units of blood (range 2-6) and 14 units (4 packs) of platelets (range 8-24 units). These requirements per day are half those of successfully grafted aplastic children.

Reconstitution after transplantation was rapid and was proved by chromosome assay in the seven patients with a donor of the opposite sex. The mean time to regain $0.5 \times 10^9$ and $1.0 \times 10^9$ polymorphs/l was 19.5 and 23 days respectively, and in excess of $40 \times 10^9$/l platelets was maintained from a mean of 28 days (range 3-83 days). The mean time spent with less than $0.5 \times 10^9$ polymorphs/l was 15.5 days. Previous children with leukaemia receiving allografts at the Royal Marsden Hospital

Table 99

SUPPORT REQUIRED AND POST GRAFT RECONSTITUTION

| Patient | Units of | | Days from graft to | | |
|---|---|---|---|---|---|
| | | | polymorphs | | platelets |
| | blood | platelets | $0.5\times10^9/l$ | $1.0\times10^9/l$ | $40\times10^9/l$ |
| CC | 4 | 12 | 16 | 20 | 14 |
| EH | 3 | 8 | 12 | 22 | 13 |
| SL | 2 | 20 | 26 | 28 | 34 |
| JG | 2 | 20 | 21 | 22 | 3 |
| GC | 3 | 20 | 15 | 18 | 83 |
| AB | 3 | 8 | 15 | 23 | 16 |
| LSF | 2 | 8 | 19 | 22 | 20 |
| TT | 4 | 16 | 19 | 21 | 17 |
| AF | 6 | 8 | 20 | 21 | 55 |
| LF | 2 | 16 | 26 | 30 | 18 |
| SA | 2 | 24 | 25 | 27 | 32 |

who survived more than 100 days with methotrexate as GVHD prophylaxis reached 0.5 x $10^9$ polymorphs/l at a mean of 15 days, 1.0 x $10^9$ polymorphs/l at day 18 and had sustained platelet counts in excess of 40 x $10^9$/l by day 22. Both rates of reconstitution are faster than that of aplastic children receiving methotrexate post graft. The children with AML who received co-trimoxazole as antibacterial prophylaxis were at no particular disadvantage in speed of reconstitution.

Metabolic effects of preconditioning and grafting

The immediate effect of 3.6 gms/$m^2$ of cyclophosphamide and 1000 rads TBI on these children was mainly a biochemichal one. Other short-term effects on the skin and the brain are those well-recognised after radiation. Long term, growth as well as endocrine effects deserve study.

Plasma amylase may reach 7000 I.U. on the day after radiation (mean 4250 I.U.) but rapidly falls and should be normal by the fourth day after transplantation. Iso-enzyme studies have confirmed the salivary glands as the origin of this amylase. No glyosuria was seen purely as a result of grafting. Plasma urate levels during marrow grafting have been normal despite no patient receiving allopurinol during preconditioning, even in those adult patients who were technically in relapse at the time of preconditioning.

The only child with elevated alanine aminotransaminase (ALT) levels pre-graft also had atypical lymphocytes and subsequently excreted cytomegalovirus. Even so, her ALT level fell following preconditioning but rose later in association with CMV excretion. Two-thirds of children showed a trivial increase ( $<$ 20 I.U.) in ALT during their transplant. Some rise in bilirubin occurred in most patients. In four this exceeded 35 $\mu$mols/l; one had a urinary infection, three had moderate GVHD with one child also receiving I.V. feeding. Alkaline phosphatase levels were not usually disturbed. All measurements were normal in nine patients but two developed unexplained levels in excess of 500 I.U., which subsequently fell. No other patient had a level in excess of 200 I.U. Half the patients received vitamin K on account of a prolongation of their prothrombin time by four or more seconds. All patients had a low serum albumin and two

with diarrhoea had a marked reduction. Both received supplemental plasma. Within the limits of the dye-binding method used most measurements were in excess of 25 gms/l. Earlier experience suggested that two-thirds of those with a serum albumin of less than 25 gms/l died, so any patient with less than 25 gms/l of albumin received exogenous replacement. If the more reliable immunological methods of measuring albumin are used, a lower level will be found.

All children had reduced calcium and phosphate levels, usually from day 5 through to day 15 or 20. In all the patients the low serum albumin contributed to the reduction in plasma calcium, but even correcting for the low serum albumin still showed a 5-10% reduction in plasma calcium. No child showed tetany or paraesthesiae and additional calcium did not assist the two who had convulsions. Magnesium levels when measured varied with the calcium. There was no connection between the 10% fall in serum phosphate and evidence of renal tubular leaks of urate, bicarbonate or glucose. No studies of parathyroid gland function following TBI have been reported.

Sodium and potassium levels were complicated by the use of gentamicin with carbenicillin or ticarcillin, a fall in both electrolyte levels showing within 48 hours of commencing these antibiotics. Withdrawing these antibacterial agents led to a return to normal over 48 hours. Levels of blood urea and creatinine were frequently low and it was only in those with diarrhoea that urea levels rose as a measure of dehydration until about the third week when most patients showed some sustained rise in blood urea. This was almost certainly due to cyclosporin-A, and blood level measurements of cyclosporin-A have now reduced the incidence of this complication.

There are few specific metabolic upsets associated with bone marrow grafting in acute leukaemia. The most consistent and specific is elevation of serum amylase during the first three days. All other changes of note may be attributed to a combination of problems. The early use of albumin, plasma and electrolyte supplements is useful along with sufficient fluid to maintain a urine output of 1 litre/$m^2$/day.

Results of transplantation in childhood AML

Six other children with AML grafted in remission at the Royal Marsden Hospital can be added to these eleven and the seventeen compared with the children suffering AML who have also been treated at the Royal Marsden Hospital but with conventional maintenance therapy. This group spans a greater age range and includes children treated from 1973. Therefore the conventionally treated patients do not represent optimal present day chemotherapy, partly due to improvements in the range of drugs and support available, but also because of a reluctance to subject particularly young children to intense therapy which was most unlikely to result in a cure. However, if children aged less than six years are excluded from those receiving conventional maintenance the age difference between the groups is reduced. Of these seventeen who received conventional maintenance therapy three are alive, one of whom is presently in relapse. Fourteen of the seventeen transplanted are alive, one of whom is in remission following relapse (table 100). The present mean survival from diagnosis for the conventionally treated children is 79 weeks compared with 91 weeks for those transplanted. The mean duration of first remission for those treated conventionally was 45 weeks and for those transplanted in first remission is at present 71 weeks. Thirteen of the fourteen transplanted in first remission were grafted before the 45 week mean duration of first remission in the conventionally treated group and currently ten of those thirteen are alive with the mean duration of first remission of this whole group being 62 weeks and the mean survival being 84 weeks. Therefore even the group transplanted before they were likely to relapse have shown an increased duration of first remission and a prolonged survival. At no point are the results of grafting in remission worse than conventional therapy.

The medians are similar to the mean results though 2-3 weeks less and with thirteen of the seventeen transplanted alive and in continued remission from before the time of their transplant, both figures should continue to increase, whereas only two of the original seventeen treated conventionally are alive in continued remission. Fourteen (82%) of seventeen children transplanted because of AML are alive a mean of 75 weeks

Table 100

CONVENTIONAL THERAPY VERSUS TRANSPLANTATION

CHILDREN AGED 7 OR MORE WITH AML (RMH)

| | Mean age at diagnosis (years) | Alive | Mean survival from diagnosis (weeks) | Mean duration of first remission (weeks) |
|---|---|---|---|---|
| Conventional n = 17 | 10 | 3 (1) | 79 | 45 |
| Transplanted n = 17 | 12.5 | 14 (1) | 91+ | 71+ |

( ) = relapsed

and a median of 74 weeks from obtaining complete remission. These results are more remarkable when it is considered that four of the transplant donors were not matched siblings. Of the thirteen patients receiving matched sibling grafts in this series, ten (77%) are alive a mean of 81 weeks after first complete remission was achieved.

One patient died of GVHD thirty-four days after his transplant, an acute GVHD death rate of 6%. One of eight alive more than a year after their transplant has chronic GVHD. Three patients have relapsed, two of whom are dead, the other in remission. One relapse at 82 weeks after grafting showed ALL and since the original diagnostic marrow was never made available and no marker or cytochemical examinations were made of the original marrow, it is quite possible that he never had AML but he is included in the series. The mean time to relapse (three patients) after grafting was 57 weeks but the inclusion of the patient who relapsed at 82 weeks makes discussing the risk of relapse at certain times difficult. Excluding this patient, the latest time relapse has been reported following an allogeneic graft for AML is 65 weeks, a point now passed by 6 of the 14 sruviving children.

Although these are early results, they are very encouraging especially as the quality of survival for all but two of the fourteen evaluable within my personal experience (table 98 ) is excellent. The two children with neurological problems may not show any improvement but this problem is not one usually following marrow transplantation and with the advent of cyclosporin-A serum assays direct toxic effects should be avoided in future. The question of cyclosporin-A affecting the handling of virus infections may be a contributing factor.

# VIRAL INFECTIONS IN BONE MARROW TRANSPLANT RECIPIENTS

## CYTOMEGALOVIRUS

### Introduction

The reports of Neiman et al (1977) and Winston et al (1979) describe the role of CMV in 140 patients who received marrow transplants on the West Coast of America. Of Neiman's 80 patients with aplasia or acute leukaemia, 43 developed interstitial pneumonitis of whom 28 died. 47% of pneumonitis cases also had CMV and of these 80% died, compared with 50% of those with interstitial pneumonitis but no evidence of CMV. CMV associated interstitial pneumonitis killed 20% of the patients successfully grafted in Seattle and 10% of those successfully grafted in Los Angeles. Combining the results from London (Barrett 1979) and Paris (Gluckmann et al 1978) there were 27 successfully grafted aplastics, of whom three (11%) died with CMV. CMV therefore exerts a considerable influence on the outcome of marrow transplantation.

To diagnose CMV infection the most useful specimens are urine, throat washings, leukocytes and serum. Sputum and bronchial washings or brushings are less useful. The most useful techniques are culture, microscopy for intra-nuclear inclusions and serology. Lung biopsy allows identification of other pathogens but is a highly invasive procedure. Monitoring every 2 weeks, Neiman et al (1977) found 46% of their transplant patients to shed CMV from some site (including at postmortem), the latest timing of the first isolate being 130 days (median 53 days). Fourteen of fifteen patients who had a four-fold rise in antibody titre had CMV isolated in culture. Sero-conversion may be affected by the transplant preconditioning but is anyway a later event than the infection even if early antibodies are sought. Culture also takes time so the search for intra-nuclear inclusions or the presence of circulating atypical lymphocytes is important, particularly as new therapeutic possibilities develop. Antibody studies are useless in the immune deficiency syndromes, but detection of CMV antigen, free or cell bound may be possible in the future.

Isolation of CMV or seroconversion does not always herald

significant disease. CMV was isolated but no pneumonia developed in 25% of those transplanted for acute leukaemia and 35% of patients transplanted because of severe aplasia. Eleven percent had CMV present in the lungs at autopsy without there being any indication of pneumonia in life. However 68% of leukaemia transplant patients and 83% of aplastics who developed lethal pneumonia shed CMV (Neiman et al 1977). Of 25 shedders (46% of leukaemia patients), 15 (60%) died of pneumonia as did 41% of the 12 aplastic shedders who constituted the same proportion of the aplastic population. Half those who shed CMV from any site after grafting in Seattle or Los Angeles died from pneumonitis.

In both series failure to seroconvert following shedding CMV was of serious portent. All those who failed to seroconvert when CMV was the sole organism obtained at lung biopsy died compared with 20% of those who seroconverted (Neiman et al 1977). Sixty-three percent of Los Angeles transplant patients with interstitial pneumonitis but no CMV seroconversion died compared to 18% of those who seroconverted (Winston et al 1979). Seroconversion is the single most important question in the event of CMV being isolated from a marrow transplant recipient.

About 54% of the adult indigenous population of London have CMV antibodies (Stern and Elek 1965). In 82 potential bone marrow donors 49% had CMV antibodies but of another 22 potential donors with tissue type HLA BW15, 73% had CMV antibodies ($p < .01$) (Pereira et al 1979). The incidence of antibody presence is therefore different within an apparently homogeneous population, perhaps as a variable expression of antibody response. If the marrow donor is an HLA identical sibling, any such HLA linked variability of expression will be constant.

Presence of antibody is taken to indicate immunity though neither this, nor the converse may be entirely true. Patients with CMV antibodies before transplantation have three times the incidence of CMV shedding after their transplant compared to those who have no complement fixing antibody before the transplant (Neiman et al 1977) but Winston et al (1979) found no such difference although they did not perform routine viral surveillance cultures. Neiman et al (1977) isolated CMV slightly more often from the recipient of marrow when the donor was CMV antibody

positive. No group has reported the incidence when marrow from CMV positive donors was given to CMV negative recipients. Fewer children (22%) shed CMV after grafting than adults (63%). Children are more likely to lack CMV antibody and to receive marrow from a negative sibling. Patients who require white cell support have a slightly higher incidence of CMV isolation (44%) compared with those who do not receive additional white cells (34%). It would seem reasonable that if the recipient and donor are CMV negative, blood products from CMV negative donors should be used.

There are no differences in the incidence of CMV infection that have been associated with sex or the use of steroids in conventional doses. Whether high dose methylprednisolone used in GVHD will affect the incidence remains to be seen but GVHD itself is associated with an increase from 34% to 57% in the incidence of CMV shedding. (Neiman et al 1977). Although the incidence of virus excretion is increased with the use of ALG, patients with grade I GVHD who did not receive ALG had a similar incidence of CMV excretion to those with grade II GVHD or greater. The incidence of CMV shedding is the same in those transplanted for acute leukaemia or aplasia but there is a difference in the incidence of interstitial pneumonitis, though not the death rate. One poor prognostic feature, apart from failure to seroconvert, was the isolation of CMV from peripheral white cells. All five patients of Neiman et al (1977) who had positive cultures from buffy coat died of pneumonitis, which is in contradistinction to the London experience where of four patients whose white cells contained CMV, none developed pneumonitis or died.

Adenine arabinoside (Ara-A) had no prophylactic value (Kraemer et al 1978) and the patients so treated had more interstitial pneumonitis and CMV isolation than the controls. Both Ara-A and interferon have marrow toxicity unlike acyclovir (Selby et al 1980). Unfortunately the in-vitro sensitivity of CMV to acyclovir is much less than herpes simplex and nephrotoxicity seems a likely accompaniment of blood levels adequate to treat CMV. Vaccines have given serological conversion without late virus shedding in normal volunteers (Elek and

Stern 1974; Plotkin et al 1976). However, it would not be wise to give those live DNA viral vaccines to leukaemia patients or their donors pre-graft. Aplastic patients are usually ill and require a transplant as soon as possible without waiting for CMV antibody responses to a vaccine. Whether killed CMV vaccines can give a useful result in these patients is not known but early work has not been encouraging. Normal human immunoglobulin has some CMV antibody and hyperimmune preparations may be available but its efficacy has never been proved. In the event of active infection, irradiated parental lymphocytes, interferon or immunological modification with thymosin might prove useful. Despite its disadvantages the present therapy is probably Ara-A until the place of acyclovir is resolved.

Clinical studies

CMV was not identified in any of the infants with SCID. Details of the CMV status of twenty leukaemia and aplasia transplant children are shown in table 101 .

Five of the twenty had CMV antibody pre-transplant and of these, two shed CMV after grafting and both seroconverted. Of five children without pre-existing antibody who received a graft from a CMV positive donor, none shed virus but two seroconverted. Of the ten without antibody who received marrow from a CMV negative donor, one shed virus and seroconverted and a further two seroconverted. Both the shedder and one seroconverter received multiple transfusions from random donors and the other patient seroconverted 80 days after going home so the infection could just have arisen unrelated to the transplant.

Of the seven patients (35%) who had evidence of active CMV infection two had no apparent problems (table 102 ). Two rejected their grafts, one following GVHD and two others developed GVHD. Two had convulsions but showed a complete recovery. Only one (J.W.) had a constellation of symptoms suggestive of CMV infection.

She received a bone marrow graft from her histocompatible 11 year old sister. Cyclophosphamide and ALG was used as preconditioning, and she was nursed in a Vickers-Trexler isolator. Eleven days after grafting she developed fever, diarrhoea, elevated hepatic enzymes and a morbilliform rash.

Table 101

CYTOMEGALOVIRUS IN BONE MARROW RECIPIENTS

| Patient | Pregraft antibody titre | Donor antibody titre | sero-conversion (day post graft) | post-graft virus isolation and site | max titre post-graft |
|---|---|---|---|---|---|
| JW | <8 | <8 | Yes (16) | Yes (53-60) leukocytes | 32000 |
| AU | <8 | <8 | No | No | <8 |
| KG | <8 | <8 | No | No | <8 |
| LP | <8 | <8 | Yes (36) | No | 64 |
| LA | 32 | <8 | ? | No | |
| MR | <8 | <8 | No | No | <8 |
| MB | <8 | + | Yes (60) | No | 64 |
| PC | 64 | <8 | Yes (32) | Yes (110) leukocytes | 1024 |
| RS* | <8 | + | Yes (18) | No | 1024 |
| GC | <8 | 32 | No | No | <8 |
| JG | 32 | <8 | Yes (42) | Yes (21-38) leukocytes then urine | >2048 |
| EH | 8 | 8 | No | No | 8 |
| SL | <8 | 8 | No | No | <8 |
| CC | <8 | <8 | Yes (120) | No | 64 |
| AB | <8 | 8 | No | No | <8 |
| LS-F | 32 | 64 | No | No | 16 |
| TT | <8 | <8 | No | No | <8 |
| AF | <8 | <8 | No | No | <8 |
| LF | <8 | <8 | No | No | <8 |
| SA | <8 | <8 | No | No | <8 |

* syngeneic graft

Table 102

PROBLEMS IN TRANSPLANT RECIPIENTS WITH ACTIVE CMV INFECTION

| Patient | Nature of infection | Problems |
|---|---|---|
| JW | Symptomatic | convulsions, chronic GVHD |
| LP | Asymptomatic | acute GVHD, rejection |
| MB | ?asymptomatic | rejection of transplant |
| PC | Asymptomatic | acute GVHD |
| RS* | Asymptomatic | none |
| JG | Symptomatic | convulsions |
| CC | Asymptomatic | none |

* syngeneic graft

Skin biopsy showed GVHD for which she was given equine ALG. Clinical improvement but slow haematological recovery followed, requiring multiple transfusions with fresh blood products.

Forty-five days after grafting, her pyrexia recurred, with mental confusion, jaundice and severe diarrhoea, but without pneumonitis. There was elevation of CMV antibody titres (table 101 ) and circulating atypical lymphocytes were present. Peripheral blood leukocytes cultured in human embryonic lung tissue were the only source from which virus could be grown.

She received adenine arabinoside 15 mg/kg/day which had to be discontinued on the eighth day because of haematological suppression. Daily cultures showed no virus growth on the eighth day of chemotherapy.

Six months later, she developed fever and mild jaundice. Her CMV IgG antibody titre was 1 in 32,000 and the serum IgG was 45 g/l. Attempts to demonstrate the virus by electron microscopy of the urine and cultures from the oropharynx and of urine, stools and white cells were unrewarded. Her symptoms resolved with eventual recovery.

It is most likely that she acquired her infection from fresh blood products, as many of these were positive on retrospective testing for CMV antibody; neither she nor her marrow donor had serological evidence of previous CMV infection. Her susceptibility to infection may have been increased by her GVHD or its treatment with ALG. Although the value of adenine arabinoside in CMV infections is not proven, it produced rapid clinical improvement without irreversible marrow toxicity. The recurrence of symptoms, with a markedly raised antibody titre, suggested that the virus was not eradicated by adenine arabinoside even though it was not demonstrable in tissue culture.

CMV seroconversion and culture

The mean time between grafting and seroconversion was 50 days (range16-120 days). Antibody titres were assayed using complement fixation techniques. In a new infection, the CMV specific immunoglobulin (Ig)M response should occur earlier than the IgG response and therefore be of diagnostic help. Table 103 shows that virus specific IgM did not rise early in four tested patients, two with presumed new infection and two

with presumed reactivation of CMV.

Table 103

TEMPORAL RELATIONSHIP BETWEEN CMV IgM AND CFT RESULTS

| Patient | Pregraft CMV antibody | Timing of rise in CMV specifif IgM titre compared to IgG CFT |
|---|---|---|
| JW | - | later |
| RS | - | later |
| PC | + | later |
| JG | + | simultaneous |

Three patients shed CMV after grafting (see below ), the mean time from grafting to CMV isolation being 61 days (range 21-110 days). Two developed GVHD and one may do when cyclosporin-A is withdrawn. All produced antibody responses and none suffered interstitial pneumonitis. Two children had pre-existing antibody and these isolations were probably reactivations rather than newly acquired infections. In all three it was only leukocytes which first provided culture evidence of CMV but two children had atypical peripheral blood lymphocytes in the 24 hours prior to the culture positive leukocytes being collected.

RELATIONSHIP BETWEEN SEROCONVERSION AND SHEDDING OF CMV

| Patient | Timing post graft of seroconversion (day) | Days when CMV shed |
|---|---|---|
| JW | 16 | 53-60 |
| PC | 32 | 110 |
| JG | 39 | 21-38 |

In retrospect two showed seroconversion before CMV shedding was detected and one shed CMV at the same time as the antibody titre rose (Table 101). The urine did not show intra-epithelial cell inclusions nor virus on culture except subsequently in only one patient. Throat cultures did not grow CMV.

## Conclusions

Although the numbers of patients with CMV infection here are small, some results are unlike those of some of the larger reported series (Neiman et al 1977; Winston et al 1979) where half those who shed CMV died as did all patients from whose peripheral leukocytes CMV could be cultured. One-third of the children who developed GVHD showed evidence of CMV infection, a similar proportion to those evaluable who did not develop GVHD. Fifteen per cent of children shed CMV after grafting and two of the three had a clinical illness with this. Pre-existing antibody made later CMV shedding more likely; seroconversion per se was not associated with identifiable clinical infection nor was marrow from a CMV antibody positive marrow graft into a CMV antibody negative recipient usually associated with identifiable problems although 40% of recipients seroconverted. Peripheral blood leukocytes were the most fertile source when culturing for CMV. Circulating atypical lymphocytes were likely to be associated with CMV and these gave an early suspicion of CMV infection but other methods such as direct immunofluorescence to identify CMV components on cell surfaces need to be developed. The use of specific IgM antibody detection was disappointing as a means to identify infection at an early stage.

## Polyoma viruses

### Introduction

Human papova viruses are usually associated with human warts but in 1971 two new strains, BK and JC, polyoma virus were identified. (Gardner et al 1971; Padgett et al 1971). The JC strain may be associated with progressive multifocal leuko-encephalopathy (PML). BK virus has yet to have any specific disease associated, but may be associated with ureteric damage after renal grafting (Coleman et al 1978) and it has been isolated from a cerebral tumour in an immunodeficient child (Takemoto and Martin 1976). Most isolates of BK virus have been from asymptomatic subjects. 5% of pregnant women excrete papova virus, usually BK though occasionally JC, in their urine (Gardner et al 1978) and excretion is particularly common if ALG is used as immunosuppression after renal transplantation

(Lecatsas et al 1973). At least 70% of adults have antibodies against these two viruses (Gardner 1973) and it is likely that just as other DNA viruses (CMV, herpes simplex and varicella/zoster, EB virus) become latent, papova viruses are latent in patients and donors. Because BK virus has been cultured from human lymphocytes(Lecatsas et al 1976) there is the possibility of transferring a primary infection in a bone marrow transplant. Just as CMV and herpes simplex may reactivate following immunosuppression, the techniques involved in marrow grafting may also activate papova viruses giving problems not previously encountered. A recent report (O'Reilly 1980) suggested that up to one-third of marrow transplant recipients shed BK virus and that this was associated with a rise in hepatic enzymes.

Clinical studies

Papova virus was found in the urine of a girl with GVHD and neurological symptoms 37 days after transplantation (Fig. 31 ). She was also infected with CMV but there was no confusion on electron microscopy (Henry et al 1977) and immunofluorescence confirmed the strain of papova virus. Since 1977 the urine of all transplant in-patient children was studied each week by electron microscopy to determine whether papova virus excretion was a common observation.

Five of eight children transplanted on account of aplasia shed BK virus in their urine (Table 104). No JC excretion was detected. All who had GVHD excreted BK virus and all who excreted BK virus had received ALG. Equine or rabbit ALG was the only common factor but was given with 20-40 mg of methylprednisolone. No high dose (500 mg/$m^2$) methylprednisolone was given. Three of eleven children transplanted on account of AML excreted BK virus. Two of the three received high dose steroids and all eleven received cyclosporin-A. None received ALG. BK virus excretion is thus not entirely an ALG effect but the result of immunosuppression in an appropriate subject and there was no regular temporal relationship to the use of either ALG or steroids.

The three children with AML were the only three patients with urinary shedding of BK virus out of 48 leukaemia transplant

Figure 31

POLYOMA VIRUS IN URINE

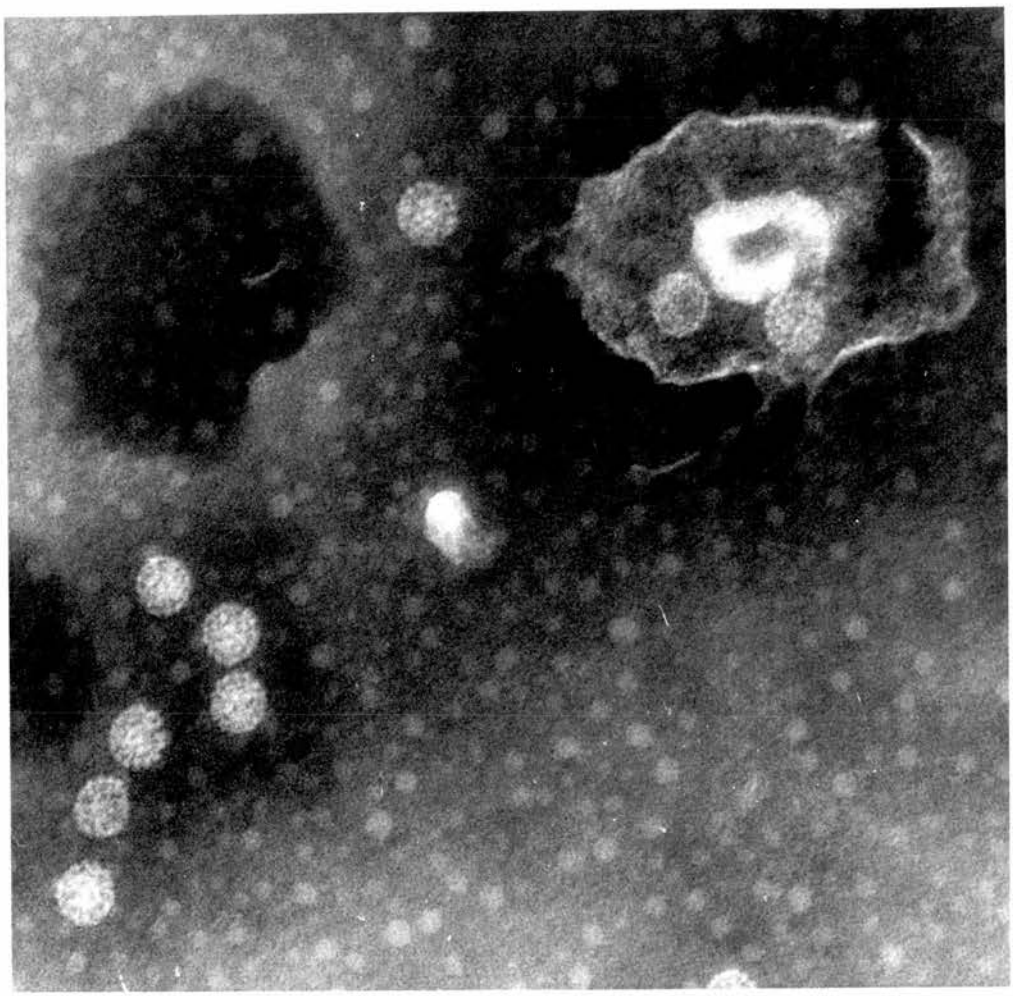


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Table 104

CHILDREN WITH BK VIRUS URINARY EXCRETION

| Patient | Diagnosis | Therapy used | GVHD | BK viruria |
|---|---|---|---|---|
| JW | AA | CY, ALG, BMT, ALG, steroids | Yes | Yes |
| MB | AA | CY, BMT<br>CY, ALG, Steroids, BMT | Probable | Yes |
| LP | AA | CY, BMT<br>CY, ALG, Steroids, BMT | Yes | Yes |
| AD | AA | ALG, steroids | - | Yes |
| KG | FA | CY, BMT, CSA<br>ALG, Steroids | Yes | Yes |
| CC | AML | CSA, CY, TBI, BMT | No | Yes |
| GC | AML | CSA, CY, TBI, BMT<br>Steroids | Yes | Yes |
| AF | AML | CSA, CY, TBI, BMT, Steroids | Yes | Yes |

AA Aplastic anaemia

CY Cyclophosphamide

CSA Cyclosporin-A

patients, including eleven less than 17 years old. Unlike those in the report by O'Reilly (1980), no particular change in liver function was seen. All were monitored weekly until discharge, a mean of 35 days after grafting.

BK antibodies

All aplastic children except one 3 year old had antibodies against BK virus (table 105) and thus had evidence of previous infection. The precise prevalence of antibodies in early childhood is not known but most children aged over four years have antibody (Gardner 1978). Any relationship between aplasia and BK antibodies seems unlikely. Those children transplanted on account of aplasia who developed GVHD had the highest pre-graft titre. The child who died of acute GVHD and the one who developed chronic GVHD both had pregraft titres of 1/10240.. It is possible that different antibody titres would identify those liable to develop GVHD although the antibody titres did not predict those who would shed BK virus (table 106 ).

It is important to realise that BK virus in the urine is common after grafting and consequently care must be taken that this virus is not confused with CMV. 'Atypical' lymphocytes were not present in the blood of those excreting BK virus. Rapid specific identification of BK by immunofluorescence should be made in the event of a neurological problem when the morphologically identical JC virus may be present. As the present recommended treatment for JC leukoencephalopathy is cytosine arabinoside (Bauer et al 1973; Buckman and Wiltshaw 1976), a drug suppressive to the emerging marrow, unnecessary treatment would be unwise, yet early treatment in the event of a true JC infection is probably essential.

Herpes Virus Infections

Introduction

Herpes simplex virus (HSV) and varicella-zoster virus (VZ) have received less attention than cytomegalovirus. However, primary infection with either virus is more likely in childhood than in adults and, in particular VZ virus must be considered seriously. Topical idoxuridine is irrelevant to systemic disease and the value of cytosine or adenine arabinoside has

Table 105

APLASTIC ANAEMIA AND BK VIRUS ANTIBODY TITRES

GRAFTED

| Patient | Age | Antibody titre pregraft | GVHD |
|---|---|---|---|
| JW | 13 | 10240 | Yes |
| KG | 12 | 10240 | Yes |
| LP | 11 | 5120 | Yes |
| MR | 15 | 1280 | No |
| AU | 14 | 1280 | No |
| MB | 14 | 640 | No |
| LA | 4 | 320 | No |

NOT GRAFTED

| | | |
|---|---|---|
| RR | 13 | 640 |
| CT | 9 | 640 |
| AD | 14 | 40 |
| JM | 14 | 20 |
| SH | 3 | 20 |

Table 106

BK VIRUS URINARY EXCRETORS AND BK VIRUS ANTIBODY TITRES
(Transplanted aplastic children)

| Excretors | | Not excretors | |
|---|---|---|---|
| JW* | 10240 | AU | 1280 |
| MB* | 640 | LA | 320 |
| LP* | 5120 | | |
| KG* | 10240 | | |

* received ALG with steroid cover

excretion status of one child was not ascertained

remained controversial. Both drugs are myelotoxic and would be used reluctantly in the early period after a bone marrow transplant. The new antiherpes virus agent, acyclovir, is not myelotoxic (Selby et al 1979) and it s probable efficacy makes it appropriate in the treatment of certain herpesvirus infections after bone marrow transplantation.

Herpes simplex

HSV can be cultured from mouth swabs taken from five per cent of adults with respiratory tract infections, and about 25% of the adult population have a past history of mucocutaneous cold sores. Forty per cent of adult febrile renal transplant recipients receiving immunosuppression excrete HSV in mouth swabs (Stalder 1978) and 26% of bone marrow transplant recipients in Seattle excreted HSV, almost all within the first 30 days after grafting (Neiman et al 1977). Twenty-five per cent of patients transplanted for AML at the Royal Marsden Hospital develop clinical HSV infections by 30 days compared with one in eight patients undergoing remission induction of AML. HSV mainly causes discomfort and interference with nutrition but generalised HSV caused two of 28 deaths from pneumonitis in 80 Seattle transplant recipients compared to 16 similar deaths due to CMV (Neiman et al 1977).

This incidence of CMV associated deaths led to an unsuccessful trial of intermittent prophylactic adenine arabinoside in a dose of 5 mg/kg/day (Kraemer et al 1978). Those treated had twice as many HSV isolations and more interstitial pneumonitis than the untreated group and the only case of clinical VZ infection occurred in the treated group. One-third of treated patients showed a drop of more than 25% in their white count.

Treatment with acyclovir

Ten of 40 transplant recipients have received acyclovir on 11 occasions on account of HSV lesions (table 107 ). All episodes were oro-facial and all virologically proven were due to HSV type I. Two patients whose lesions were extending despite thrice daily topical idoxuridine (IDU) healed in 4 days, and nine episodes (82%) were much improved on the third day. One (BM,2) was a treatment failure, but her mouth lesions could have

Table 107

HERPES SIMPLEX INFECTIONS
IN MARROW TRANSPLANT PATIENTS

| Name | Weeks post Graft | Dose of Acyclovir mg/kg for 5 days | Nature of response |
|---|---|---|---|
| MR | 3 | 5 mg tds | chin crusted by day 3 mouth healed by 7 days |
| PD | 3 | 7.5 mg bd | pain improved by day 2 lip crusted by day 3 |
| BM[1] | 2 | 5 mg tds | mouth healed by day 4 |
| BM[2] | 4 | 10 mg tds | no effect until Flagyl given on day 4 |
| RR* | 2 | 5 mg tds | mouth healed in 4 days |
| BT | 2 | 5 mg daily | chin crusted by day 3 mouth much improved by day 3 |
| GB | 2 | 5 mg daily | mouth healed by day 3 |
| CC* | 12 | 5 mg daily | lip crusted by day 3 |
| AI | 3 | 5 mg tds | mouth healed by day 4 |
| DB | 2 | 5 mg tds | pain improved by day 3 mouth healed by day 6 |
| LS-F | 4 | 5 mg tds | pain much improved on day 2. healed by day 6 |

*lesions were extending in the presence of IDU

been due to neutropenia with anaerobic infection because they responded as the neutrophil count rose and oral metronidazole was given. Isolation of HSV from the mouth does not necessarily mean that it is the cause of oral ulceration, but it is compelling that it must be playing a part even in the presence of neutropenic mucositis.

Varicella-Zoster

Varicella/Zoster (VZ) infections are common and 44% of marrow transplant recipients in Seattle suffered clinical VZ, half during the first six months after grafting. (Atkinson et al 1979) One of 40 patients had a second attack.

There was no particular association with age or sex of the recipient, the original diagnosis or the nature of the transplant preconditioning regimen and unlike other infections there was no association with GVHD. The only association was with an absent cutaneous response to DNCB indicating depressed cell mediated immunity. Gallagher and Meriden (1979) found an absence of in-vitro cellular responses to VZ virus in patients with prolonged zoster. The in-vivo presence of VZ antibody was irrelevant.

Two of eighty marrow transplant patients have died of VZ at the Royal Marsden Hospital and one of 40 transplant recipients at Westminster Hospital. None received prophylactic varicella immune globulin (VIG) but all transplant patients now receive this every fortnight from discharge until 14 weeks after transplantation.

Ten of twenty-nine transplant recipients surviving within the past year to between 2 and 15 months after grafting have developed VZ infection. In eight patients zoster was localised to one or two dermatomes (table 108 ). One patient with disseminated zoster had suffered three episodes of pneumococcal pneumonia, suggesting that immune reconstitution was incomplete. Two of the nine patients had no associated pain. Three were receiving VIG at the time of their eruption and six of the nine developed zoster within the first six months after grafting. Half were also still receiving cyclosporin-A.

All nine received acyclovir. One (S.L.) was a treatment failure. He continued to produce new lesions within the same

Table 108

## ZOSTER IN MARROW TRANSPLANT RECIPIENTS

Localised

| Name | Months post graft | Dose of Acyclovir mg/kg for 5 days | Duration of rash pretherapy | Nature of response |
|---|---|---|---|---|
| SL[1] | 5 | 5 mg tds | 1 day | new lesions in same dermatome for 7 days |
| AW[1] | 3.6 | 4 mg daily | 1 day | pain improved by day 2<br>no new lesions |
| CC* | 5.5 | 8 mg bd | 6 days | new lesions day 1 only |
| SC | 9.3 | 5 mg tds | 1 day | pain gone by day 2<br>new lesions day 1 only |
| NP[1] | 2.5 | 4 mg daily | 2 days | no new lesions<br>itch gone at 36 hours |
| SB | 5.7 | 5 mg tds | 3 days | no new lesions<br>pain gone by day 2 |
| PN[1] | 2.8 | 5 mg tds | 5 days | no new lesions<br>pain relief |
| BM | 6.2 | 10 mg daily | 4 days | no new lesions<br>much improved on day 4 |

Disseminated

| | | | | |
|---|---|---|---|---|
| KC | 11 | 5 mg tds | 14 days | new lesions day 1 only |

* no pain

[1] also received VIGG

dermatome and also required reduction in the dose of acyclovir because of azotaemia. He was the only patient also receiving steroids because of a haemolytic anaemia. Those of the remaining eight patients who produced new lesions did so only during the first 18 hours after starting acyclovir. None has had a second episode of zoster. Pain relief was also achieved but owing to the free use of analgesics, this effect was not entirely due to the acyclovir. Complications developed in three patients, one due to extravascular leakage of acyclovir which has a pH of 11 and two patients showed a transient rise in blood urea that could not be otherwise attributed. This may be due to acyclovir precipitating in the renal tubules when there is renal clearance of the plasma peak following bolus injection. Giving similar or higher doses of acyclovir over 1 hour as an infusion has recently eliminated this problem. Five patients received acyclovir while their graft was taking and no disturbance of the normal pattern of graft-take was seen.

Conclusions

In marrow graft patients acyclovir would appear to be beneficial and not myelotoxic. Since the natural history of both VZ and HSV infections is so variable, it remains uncertain that acyclovir shortened the natural history of these infections or was the cause of the response seen in 87% of VZ and 82% of HSV infections. Firm conclusions await a controlled trial, as other anti-viral agents were greeted with early enthusiasm. However one disseminating zoster and two HSV infections which were extending despite IDU, were rapidly controlled. In a wider uncontrolled report of 92 patients treated with acyclovir (Watson 1981) 10% of patients were considered to have shown no response to therapy.

MARROW GRAFTING IN CHILDHOOD - THE PRESENT NEED FOR FACILITIES

The relevance of marrow transplantation is limited only by the graftable constituents of the transplant. Engraftment of erythrocytes, leukocytes and platelets can be ensured, so conditions involving any quantitative or qualitative defect of the stem cells or their progeny may be amenable to bone marrow transplantation. Grafting of competent cells may be appropriate in those inborn errors of metabolism expressed in the circulating haemopoietic cells until other methods of enzyme replacement are developed. GVHD, the unacceptable effects of present preconditioning regimens at certain ages and the dangers of infection do not limit the relevance of grafting, but limit its application.

In the more experimental fields the natural history of each condition must be weighed against the immediate outcome of a failure to achieve engraftment. This does not necessarily mean death as unless TBI has been used, autologous reconstitution may occur and this can probably be ensured by previously storing the child's marrow and reinfusing it if engraftment fails. In addition, the family attitudes will also affect the decision to attempt a transplant, some parents much preferring that a radical cure was sought, especially if they already have experience of the disease. However in the diseases where there is a considerable body of experience to draw on, i.e. SCID, severe aplasia and acute leukaemia especially in second or subsequent remission, such theoretical considerations do not apply and the statistical facts of their child's particular circumstances can be discussed with the parents, with the caveat that no-one can know how their particular child will fare.

A major limiting factor at present is the need for a donor compatible at the major histocompatibility loci. Such a sibling is available for less than 25% of possible patients. About 15% of patients engrafting after receiving an MHC identical sibling marrow died of GVHD before the advent of cyclosporin-A, yet attempts to develop more stringent matching criteria would further reduce the number of patients with a donor. However, encouraging results are emerging in the use of cyclosporin-A in preventing death from acute GVHD and in addition alternative techniques such as stripping the infused marrow of T and pre-T

lymphocytes by pre-incubating the marrow with steroids, lectins or monoclonal antibodies are developing and need evaluation.

GVHD prophylaxis with combined cyclosporin-A and methotrexate has prevented fatal GVHD in children (and adults) receiving a marrow transplant from a donor who only shared one haplotype with the recipient. Almost all children who are candidates for a transplant will have an available parent who must share one haplotype with their child and so we may shortly see not only a continuing reduction in the risk of fatal GVHD following matched sibling transplants but also a tripling in the number of children who have possible donors. I therefore foresee a wider practical availability of marrow transplantation for children of almost all ages, until that is, new approaches, both scientific and social, replace marrow grafting as a therapeutic option.

The indications for bone marrow transplantation can be divided into those in which the technique is of proven value and those in which it would be experimental but may be useful. Table 109 shows the inborn errors that may be considered in this manner and their likely incidence in the United Kingdom each year. Table 110 shows acquired haematological and neoplastic diseases with the incidence derived from the OPCS report of 1978. Although the total of 470 children each year (tables 109 and 110) includes 140 children (table 109) in whom the procedure would be experimental, it nevertheless includes 330 children in the groups where proven success has occurred and of these no less than 213 children have diagnoses sufficiently common that comparison between those grafted and those treated conventionally shows that those grafted are definitely not at a disadvantage compared to those treated conventionally although a longer follow-up of the transplanted children is required to evaluate the long term gain.

The number of deaths in table 110 are taken from the mortality data reported by the Office of Population, Census and Survey (1978). Seventy percent of children with ANLL will achieve a first remission but 10% of these will probably be less than three years of age and therefore one might be disinclined to use TBI. A similar reduction due to age will apply to the

Table 109

## EXPECTED ANNUAL INCIDENCE OF CHILDREN WITH INBORN ERRORS TREATABLE BY BONE MARROW TRANSPLANTATION

| | New patients/50 million population | |
|---|---|---|
| | PROVEN | EXPERIMENTAL |
| **Causing IMMUNODEFICIENCY** | | |
| 1. Severe combined immunodeficiency (SCID) | 35 | |
| 2. Pure T-cell deficiency | 5 | |
| 3. Wiskott-Aldrich syndrome | 10 | |
| 4. Errors intrinsic to phagocytes | 50 | 50 |
| 5. B-cell deficiencies | | 10 |
| 6. Stem-cell aplasia | 2 | |
| **Causing DEFECTS OF DNA REPAIR** | | |
| 1. Fanconi's aplasia | 10 | |
| 2. Ataxia telangiectasia | | 5 |
| 3. Xeroderma pigmentosa | | 5 |
| **Metabolic errors EXPRESSED IN LEUCOCYTES** | | |
| 1. Mucopolysaccharidoses ) | | |
| 2. Lipidoses ) | | |
| 3. Urea cycle enzyme deficiencies ) | | |
| 4. Maple-syrup urine disease ) | | 70 |
| 5. Glycogen storage disease due to branching enzyme deficiency ) | | |
| 6. Others (Tay-Sachs, Galactosaemia, etc.) ) | | |
| OSTEOPETROSIS | 8 | |
| | 120 | 140 |

partly Stanbury et al (1979)

Table 110

EXPECTED ANNUAL INCIDENCE OF CHILDREN WITH HAEMATOLOGICAL/ONCOLOGICAL DISEASE TREATABLE BY ALLOGRAFTING

| Cause of death (OPCS 1978) | Number of deaths (0–14) | Candidates for transplant (see text) |
|---|---|---|
| Acute non-lymphoblastic leukaemia | 60 | 38 |
| Acute lymphoblastic leukaemia | 173 | 140 |
| Other lymphoid malignancies | 84 | 19 |
| Other diseases of the blood | 42 | 16 |
| Total | | 213 |

90% of children who achieve an adequate second remission in ALL and an additional 10% may not be suitable for TBI because they had received prophylactic cranial irradiation within the previous few months. Perhaps half of these could be transplanted in a third remission. Half of those with 'other lymphoid malignancies' had Hodgkin's disease and of those with lymphosarcoma perhaps half (less the youngest ten percent) could be eligible. Deaths from other diseases of the blood accounted for 42 children in 1978, of whom at least the twenty who suffered aplastic anaemia, agranulocytosis or thrombocytopenia could in theory have been transplanted, but 20% (at least) would not have been in sufficiently good clinical condition for this to be feasible.

Of the 330 children (tables 109 and 110) with diagnoses for which successful marrow grafting has occurred, statistically one in four of these children should have a compatible sibling donor. Particularly in the case of infants, this sibling may not yet have been born which reduces the changes of about 60 children having a matched sibling donor to one in eight. Although a few children will have a compatible non-sibling family donor, it is likely that of the 330 children per annum in whom transplantation could be indicated, only 70-80 each year would be in a position to receive a matched sibling bone marrow transplant. This figure of course will change if the more recent ALL treatment regimens (UKALL 8) prove more effective in giving long term disease-free survival than the earlier regimens. The present short-fall of about 250 children, almost all of whom will have a one-haplotype identical parent available as a donor, demonstrates how the demand for facilities will change if methods to prevent GVHD following a one-haplotype marrow transplant are successful. Even if in-vitro treatment of autologous marrow in leukaemia proves effective, there will remain the demand for marrow transplant facilities.

Combining the immune deficient infants, the patients with severe aplastic anaemia and the children with ANLL in first remission and acute lymphatic malignancy in second or subsequent remission (tables 109 and 110), the total of between 70 and 80 children each year who have a matched allogeneic sibling out of a total population of 55 million will mean that one marrow

transplant in childhood should be expected each year per 730,000 total population, and if one-haplotype identity is acceptable, one childhood allogeneic marrow graft per 180,000 total population may be envisaged.

The almost certainly underestimated data of adult deaths from acute leukaemia and aplastic anaemia (OPCS 1978) under the age of 50 years suggests that about 90 adults in the United Kingdom per annum will be candidates for grafting and have a matched sibling. Overall in the United Kingdom therefore we should expect about 160 matched sibling allografts each year i.e. 1 per 340,000 of the total population, a figure which takes no account of 'autografting' techniques, pseudo-syngeneic second transplants or of patients coming to the United Kingdom under the terms of our membership of the European Economic Community.

Adult patients with AML in remission spend a mean of 38.4 days as in-patients while receiving their transplant (Kay et al 1980). Patients transplanted on account of severe aplasia will require slightly longer in hospital purely as a result of their graft as reconstitution is less fast. Similar data for children is shown in table 111 which indicates that to support the present requirement for matched allografting, in theory 21 beds would have to be in continuous use throughout the United Kingdom. It is more likely that 30 designated beds would be required and these should be in six separate units, each with between four and six beds and each receiving from a population of between seven and ten million. No centre should then experience less than twenty transplants each year. However the small number of infants will definitely not fit into such a scheme and it is doubtful if even older children should be in an adult marrow transplant unit <u>providing</u> the facilities and skill required for them to have a successful outcome exist in a Children's Hospital. It is readily apparent how these requirments would escalate if donors other than matched siblings prove acceptable.

The additional cost of such a matched sibling allograft programme will not in fact be great as these patients are requiring care anyway and numerous unquantifiable factors come into play, including future earnings, tax paying ability and reduced future medical costs in an inflationary economy. Even

Table 111

DAYS IN HOSPITAL EACH YEAR FOR PATIENTS ADMITTED FOR A MATCHED SIBLING MARROW ALLOGRAFT

| Disease | Days as in-patient required for graft | No. of patients p.a. in U.K. (page 295) | Total in-patient days per year |
|---|---|---|---|
| Adults | | | |
| Acute leukaemia | 38.5 | 70 | 2695 |
| Acute aplasia | 45 | 20 | 900 |
| Children | | | |
| Acute leukaemia | 42 | 50 | 2100 |
| Acute aplasia and similar | 59 | 16 | 944 |
| SCID and diseases of infancy | 84 | 9 | 756 |
| TOTAL | | 165 | 7395 |

so,transplantation of leukaemia patients costs about twice the average bed day for each hospital (Kay et al 1980). Similar care for children transplanted for acute aplasia cost 2.7 times the average bed day cost at Westminster Children's Hospital and infants with SCID cost 1.8 times the average bed day. On this basis, a matched sibling transplant programme will cost about 2.16 times the average bed day, a total of £1.6 million per annum for the 165 patients in table 111, an average of £10,000 each.

As far as we can tell,at least 60% of the leukaemia patients transplanted in remission have such a prolonged disease-free survival, they may well be cured. Survival rates for the transplanted aplastic patients and the infants with severe combined immune deficiency are not worse than 40% and 55% respectively. On this basis, the cost of a successful outcome using matched allogeneic siblings in childhood transplantation can be derived. In acute leukaemia it will be £14,000; in severe aplastic anaemia £39,000 and in severe combined immune deficiency each successful outcome will cost £27,500.

# APPENDICES

1. Tape/slide for the operation of Vickers-Trexler isolators.
   (for tape - see end folder)

2. Germfree delivery of infant with possible severe combined immune deficiency.

3. How I'm still alive by A.U. age 13 years.

APPENDIX ONE

## TAPE/SLIDE FOR THE OPERATION OF VICKERS-TREXLER ISOLATORS WESTMINSTER CHILDREN'S HOSPITAL

There have been a few minor changes since this tape slide was made so not everything in the isolator you will be using upstairs is exactly the same as described here. However, the differences are only minor ones. Slide one.

This first slide shows a cut-away drawing of an isolator tent, inside which you can see a patient and her bedding. The PVC envelope is kept in place by metal rods, and the tent kept at a positive pressure by a motor which blows air into the isolator. If any holes occur in the PVC, air will pass from inside to outside of the isolator, not the other way round. The alphabetical letters indicate various parts of the isolator equipment:

A) shows the control rod. This connects the PVC envelope to a valve. The degree of inflation of the isolator is thus automatically controlled. B) indicates the port connecting the patient envelope where two nurses are working, to the supply envelope where one nurse is working. When the two envelopes are separated, the patient enters the isolator through this port. C) shows part of the unit which filters the air in-coming from the blower. This filter will remove particles down to a size of 0.3$\mu$, and will thus trap bacteria and dust particles. Although viruses are smaller than this, they are usually adherent to dust particles and will also be trapped. D) indicates a hatch, through which items may be introduced or removed from the isolator whilst it is in use. The newer isolators have better designs of hatch, but the principles remain the same. E) shows the air supply for one of the nurses working in the isolator. She is in a half-suit which is also shown as F). These half-suits are large invaginations of PVC which have a firm, clear visor. Staff may work so to speak 'in the tent' yet still remain outside. The air supply E) is necessary, and flows to an air-waistcoat which is hung round the neck, ensuring a supply of air to the face, chest and

arms of the person working in the half-suit.

The second slide shows a diagramatic view, with the patient inside the isolator. The PVC is marked in red and the solid structural parts are black. The mattress is cross-hatched. A standard 6 foot (182 cm) NHS bed is outside the PVC, and the mattress is tucked in a pocket in the bottom of the envelope. This pocket allows normal bed-making with tucking in of sheets and blankets. There are filters where air enters the isolator and also where air comes out. The filter on the air exit is necessary because if somebody working in a half-suit steps backwards, some suction will result and ward air could be drawn into the isolator. The filter ensures that any air drawn back is sterile. The PVC of the bed envelope and the supply envelope is firmly joined to the main port by sticky tape and an elastic band. The base of the supply isolator is attached to the bed to avoid accidental separation of the isolators.

The third slide shows a bed isolator in operation. You can see a patient in the isolator, a nurse working in the supply isolator, and part of the air filter system in the top corner of the tent. The blower, control valve and air flow guage are also shown. The blower will give a maximum flow of about 50 cubic feet (1400 litres) per minute with the filters in place.

The fourth slide shows the cot isolator. This isolator is based on exactly the same principles but is smaller and there are no half-suits. Instead, there are eight sleeves with gloves set in the wall of the isolator. This is a standard NHS 4'6" (137 cm) cot, with the adjustable back swung upwards out of the way. The supply isolator at the far end is exactly the same as for the bed isolator.

The fifth slide shows a transit isolator. This is placed on a trolley and has no firm structural support; it is kept inflated by a small battery or mains operated blower. We use this isolator for transferring a child from one hospital to another and have given radiotherapy to a child in this type of isolator. The child can be transferred from the bed isolator to the transit isolator without breaking isolation. Isolators are in use in many other countries and the next slide, slide six,

shows an isolator in the Hôpital des Enfants Malades in Paris. The principles are very much the same. For this infant there is a sleeping area on the right and a play area on the left.

The seventh slide shows part of an isolator in Texas Children's Hospital, Houston. This boy who is now nine years old has been in a sterile environment since birth and has a bespoke perspex isolator with living, sleeping and playing areas. There are sleeves in the walls through which the doctors, nurses and parents attend to the child's needs.

The eighth slide shows one aspect of the preparation of items for sterilisation. As everything which enters the isolator must be sterile, gamma irradiation, autoclaving or chemical disinfection is used as appropriate. The isolator itself if sterilised with Milton. This slide shows the heat-sealer for nylon or plastic bags. Items for autoclaving are packed in autoclave bags. Both these methods require the object to be double-wrapped. The reason for this will become clear when we describe the procedure for entering items into the tent. (Slide 9) Some strange things happen during the sterilisation process - some glass goes black when irradiated, and so the child must get used to having red tomato ketchup coming out of a black bottle. The little packet in the foreground was blackcurrant syrup, which lost all colour when irradiated to 2.5 megarads. (Slide 10) Some plastics change their colour, and some become brittle after repeated irradiation.

The next few slides show some aspects of the isolator itself. Slide 11 shows a pre-filter protecting the main exit filter. This pre-filter is changed every three or four days because it becomes clogged with fibres released during bedmaking. If the main exit filter was not protected in this way, then it would soon become blocked and reduce the airflow.

Slide 12 shows an oxygen monitor. Sterile oxygen is available in the isolator. The sensor in the centre right of the slide has been sterilised and the lead passed out through the tent wall and firmly taped in place, thus continuous oxygen monitoring is available. <u>Do not use electrical equipment inside the isolator when giving oxygen.</u>

Slide 13 shows the electric plug board, with several electric flexes coming out of the isolator. The flexes

(without plugs) have been irradiated and passed aseptically into the isolator. The flex is pulled out of the isolator through a small cone to the required distance, and firmly taped in place. The flex cannot be allowed to return inside, as it is obviously now unsterile. A plug is then attached and the electric equipment used normally. The other item in this slide is a sleeve set in the wall. (Slide 14) This sleeve fits inside the refrigerator. Thus items which are in the isolator may be passed, still within the sterile area, 'into' the refrigerator and so sterile cold drinks are available.

Slide 15 shows a glove-sleeve low on the wall of the isolator so toys may be picked up from the floor without the nurse having to enter a half suit. The isolators usually have 3 half-suits and 9 glove-sleeves.

Slide 16 shows some of the electrical equipment inside the isolator. There is a toaster, an automatic kettle, and a fan which can blow either hot or cold. There is also a small cooker in which meals may be heated. The bread on top of the toaster has been irradiated and is sterile.

Before entering items, the nurses turn a hand valve so as to give the maximum air flow, put on masks, and wash their hands with Hibiscrub (Chlorhexidine). (Slide 17) The area around the entry port is cleaned with Hycolin. The port is opened, and, because of the positive air pressure within the isolator, a flow of sterile air emerges. (Slide 18) <u>In this flow</u>, the outer wrapping of a pack is torn open, and the item still enclosed in the inner wrap is then grasped (slide 19) either with a hand or (slide 20) with Cheatles forceps and lifted into the isolator by the nurse working in the supply isolator glove-sleeves. The hatch is then closed. Glass can be surface-sterilised using Milton. (Slide 21) Following total immersion in Milton for 30 minutes the Milton tank is presented below the entry port and in the air-flow when the port is opened (slide 22) the nurse working in the supply isolator can reach through with Cheatles forceps and lift the item into the isolator. The hatch cover is then closed. After two or three objects have been entered, there is a pause to allow the pressure in the isolator to build up again. Ethanol is kept readily available in the

isolator in case contamination occurs. The new Mark 3 isolators have a side entry hatch but the principle remains the same.

Slide 23 shows a diagramatic view of how rubbish is removed from the tent. A bag is fitted over the exit port from the inside of the isolator and held in place with an elastic band. When the bag is full and once again from inside the tent, a new bag is fitted over the old bag. The elastic band has already been moved so that it holds only the new bag. The bag full of rubbish can then be carefully eased off from outside the tent (slide 24). This means the bag has been changed without breaking isolation. Large quantities of water, such as bath water, are removed from the tent by a Matburn sucker (slide 25). A small hole is made in the exit port bag with scissors from outside the tent. Sterile rubber tubing is passed out of the isolator through this hole and the bath water is then aspirated using the Matburn sucker with two two-litre jars in series. (Slide 26) After aspiration, the rubber tubing is pulled right out of the tent and the hole sealed using a pair of forceps. The forceps are removed before the rubbish bag is finally thrown away. Objects can also be dropped out of the entry port, to be caught by a waiting hand.

Slide 27 shows a different system, in use with the cot isolator. There is no exit port bag, but an exit shute, which is tied at top and bottom. The top tie is undone and rubbish passed into the shute. The top tie is then tightened and the bottom tie undone. The waste then falls out, but if the patient also has a communicable infection, then the bottom tie can be opened into an appropriate receptacle which will reduce the risk of cross-infection to the rest of the ward.

The next slide shows an infant being weighed in the large plastic bag set in the side of the cot isolator. The baby is inside the isolator, but the scales are outside. In a similar way, this bag can be used for x-raying if the x-ray plate is placed on a table. The infant lies over the x-ray plate and the x-ray tube can then shoot vertically downwards.

Slide 29 shows a drug being checked and drawn up inside the cot isolator. The ward sister who is checking the drug is wearing her normal clothes and is standing right beside

the isolator. Slide 30 shows a tube-feed inside the isolator. Almost all the equipment we use in the isolator is standard National Health Service issue.

The next five slides show how to enter one of the half-suits. First you remove any sharp badges or rings, then wash your hands. The air waistcoat (slide 31) is slipped over your head and the velcro strap secured round your waist. Your arms are placed inside retaining loops. This waistcoat will blow air over your face and arms. (Slide 32) You crouch down and fit the head piece over your head. (Slide 33) You then shuffle forwards "into" the isolator. In this slide you can see the clear visor. You place your arms into the sleeves of the half-suit. There are four sleeves on each half-suit, with usually two gloves of size $6\frac{1}{2}$ and two of size 8. This allows for different hand sizes. (Slide 34) You stand up and give yourself a 'shake' to rearrange the plastic in the most comfortable manner, (slide 35), and you are now able to help the patient in the isolator. All the procedures are easily taught to the most junior nurse and also to the parents.

The next series of slides show aspects of the patients and their relatives. (Slide 36) If the patient is well enough, then it is an excellent idea for him to clean the isolator himself each day. In this slide Martin is giving his isolator its daily clean. You can see he is wearing his normal everyday clothes, which have been sterilised in the autoclave. In the next slide (slide 37) he is playing draughts with one of the nurses, who is sitting on a chair while wearing one of the half-suits. The draughts set has been gamma irradiated and is inside the isolator. In slide 38 Martin is using a sterile wrapped movie-camera inside the isolator, so we outside the isolator will be able to 'see ourselves as others see us'. Slide 39 shows Martin playing cards with his father. Slide 40 shows a different use for the glove-sleeves. In the same way that we use them by reaching in, the patient can reverse the sleeve so that he can reach out of the isolator, to adjust a television, radio or use a tape recorder. Slide 41 shows that school work can be continued.

The final slide, slide 42, shows a baby being held by its mother. Mother is sitting outside the cot isolator using the sleeves, and cradling the baby in her arms. She is not

wearing a gown, cap, mask or suchlike which are usual in reverse barrier nursing. In other words, mother is appearing to her baby as she would do normally and the infant can see all her expressions and hear her voice. In addition there is transmission of body contour and warmth through the plastic.

Although there is little doubt that this is extreme isolation in bacteriological terms, this form of isolation does not isolate the patient from the environment in the way that cubicle nursing may do. For long periods of isolation our nurses prefer the isolator tent and feel less separated from the patient than when they are using cubicles for reverse barrier nursing. They like the reliability of the isolator and are convinced that the children benefit from being in an isolator within an ordinary open ward rather than being shut away in a cubicle.

We are very grateful for the dedicated nursing of all our bone marrow transplant patients by the nursing staff of Westminster Children's Hospital, London, and we also are grateful to the Elizabeth and Andrew Bostic Fund, a Registered Charity, which paid most of the specialised costs involved. Many further details of our isolator practice, including sterile foods available, techniques for sterilisation of equipment and how to manage the isolators from day to day are available in the Operation Manual which we have written.

J.G. Watson

APPENDIX 2

## "GERMFREE" DELIVERY OF INFANT WITH POSSIBLE SCID

Delivery At Westminster Hospital by vaginal route with vaginal decontamination using Betadine. Infant to be passed in sterile drapes to paediatricians. As few people present as possible, all wearing gowns, masks, gloves, overshoes and hats. Room and "furniture" to have been freshly cleaned with hycolin.

Resuscitation Such immediate measures as required will be given. The Resuscitaire will be covered with sterile drapes. Sterile LF32 filters will be available for oxygen or air lines. Procedures will involve as sterile methods as practical.

Transfer to Westminster Children's Hospital In normal double humidity Vickers 59 incubator. This will have been cleaned by Nathan ward staff using hycolin in spirit, lined with sterile drapes and sealed. Positive pressure air will be provided by a 5 cfm blower with twin bleeds and sterile microflow LF 40 filter. Transfer will be by emergency ambulance and pressure maintained using either cylinder air supply with LF 32 filter or battery operated 2 cfm blower. Staff will observe appropriate sterile precautions.

At Westminster Children's Hospital Transfer to fresh 'sterile' incubator in Nathan ward within a specially cleaned cubicle with properly closing door and windows. The child will be bathed and observed. Positive pressure air flow to continue. Cubicle reverse barrier nursing techniques to be followed.

Use of isolator Transfer will be effected electively after results of markers are known, and usual nursing care instituted. Use normal patient entry technique.

Microbiology

Mother
1. pre-delivery swabs for bacteriology, mycology, (Stewarts medium) and viruses (Hanks solution) from vagina and stool to be sent.
2. post delivery HVS for bacteriology and mycology

Child
1. swabs from external auditory meati, vagina, mouth and nose after entry into incubator

2. swabs from ears, nose, mouth, axillae, groins, vagina, throat and umbilicus at 12-24 hours of age.
3. thereafter swabs from all usual sites two times/ week until stable, thereafter as per schedule.

Drug therapy Vitamin K to be given at WCH.
Surface and orifice decontamination will commence at +2 hours but no gut decontamination

Feeding Initially 5% dextrose (sterile) then pasteurised breast milk (if available).
Otherwise SMA, or similar (prepacked, surface sterilised) and sterile teat.

Immunological assessment T and B markers (15 mls in no-beads lithium heparin) must be sent as soon as possible, perhaps from cord blood depending on time and day of delivery. Results (provisional) available in 4 hours, and firm results in 24-36 hours. Immunoglobulins (3 mls clotted). Full blood count (urgent lymphocyte count).

If abnormal: HL-A typing and family MLR's to be set up, when repeat tests at 48-72 hrs.

If normal: repeat at 48-72 hours. If these are normal, use fresh breast milk.
When showing an acceptable bacterial content, discontinue barrier techniques.

An incubator will be ready from 35 weeks gestation. An isolator is available. Cord blood must be saved in preservative free heparin (Nathan ward or Haematology Dept.) and put in non-frozen part of fridge. Use cannula attached to placental end of umbilical vein to obtain blood (from placenta). Bleed infant only if necessary.

APPENDIX 3

## HOW I'M STILL ALIVE

by

A.U. (age 13)

I opened the door. It was 10 a.m. Dad had got me up to go to the doctor's. I felt it was all a waste of time, after all the bruises and being bumped on my birthday in July and looking pale. He finally decided it was the doctor's for me. I waited about ten minutes and then went in. He said that I looked pale. My dad explained and the doctor said to go up to Queen Mary's Hospital. We went outside and waited for a bus. It was about forty minutes before one came and even then it was a little walk up to Queen Mary's in Roehampton. When I got there we had a little wait in the Out-Patient's Department. In the check-up room I had about four blood tests and then the doctor decided I should stay in 'H' ward. I was wheeled to it and was put in a bed and turned the T.V. on. After about 25 minutes I was transferred in to a little room. This little room was just the same as a mini-house really. It had a T.V., a sink, a bed and a couple of chairs. I got into bed: just to add to my hopeless attitude the T.V. started to go haywire. While the T.V. was going mad I fell asleep, slowly, but I fell asleep.

At about 5 p.m. my mum, dad, sister, brother-in-law, all came to see me. Before coming into my room they all put hats on, long aprons that went down to their knees and a mask. I thought, "This is odd". My mum and dad were first in. They looked a bit downhearted. "Hello love!" said my mum. My dad gripped my hand tight.

"When will I be coming out of here?" I asked.

"In about three weeks," said my dad looking a bit lost.

After about half-an-hour my mum and dad went out. Sue and Graham waved and then some doctors came along. I looked away, fearing something. I don't know why. I looked back to see the doctors talking to my mum and dad. Mum turned away, put her head in her hands and got upset. When the doctors had gone away, my mum put her head in the door and said, "Bye, Andrew!" All my hope of getting out of the hospital tomorrow had gone and

when I saw mum walking down the corridor, starting to cry, I thought three weeks was a long way off and would I come out alive?

I woke up the next day and started to read some comics that I had found in the drawers. After about half-an-hour some doctors came. The head doctor was a bit strict with me and I was really scared. He asked me if I had sniffed any glue, gas or paint. I said "No." Then he said I was being transferred to Westminster Children's Hospital. When my dad came I told him what they had said.

"Don't worry. We minster's a world famous hospital."

"But I was just getting used to this hospital."

My Uncle Pinky (his nickname) came in the morning. He explained that Westminster was near Stamford Bridge where he lived and he would be able to come and see me and bring Shoot, a comic. At about three o'clock they wheeled me to an ambulance and I was driven to the hospital. The cubicle there was much like the other room I had been in. I settled down just before four. I had to have a bone-marrow test. I had to have a half-inch needle put into my waist. The needle was pushed in the bone at the waist. It was very painful. After about thirty minutes pushing and pulling he pulled out the needle and there was blood all over the place. He squirted the blood into some little containers. I couldn't move for about an hour because the needle had sent all the bone numb and it hurt. The night wore on and I fell asleep. About two days later I was excited because my brother Peter was coming over from Vancouver, Canada. The day he came was Friday and we played cards all day using counters for poker. After the weekend I had more blood tests to be matched with Ena's blood from her bone marrow. They were successful and my mum cried for joy. The doctors said I would be going in a plastic bubble on Thursday and on Thursday morning I would be having an operation to have a tube put in my neck.

Wednesday night, Susan, one of the nurses came in and asked if anything was worrying me. I said the tube was worrying me. She said,

"No need to worry. You'll be given an antispectic through a needle."

In the morning two nurses came and injected me with a needle. It would make me go to sleep. I was still awake but drowsy and before I knew it I was being wheeled along to the theatre. I was sick about twice on the way. The needle pricked into my neck. The pain was terrible but it eased off and went numb. I heard scissors cutting into my neck. I started to cry. I gripped Susan's hand tight. About thirty minutes later it was over. It was sewn up and a plaster was put over it. Later I was wheeled to the bubble and put through it by a hatch. The bubble isolates you from germs. It doesn't have all that living space. Inside there is a bed, a drawer and an unusual kitchen. The kitchen is very small and is at the end. There is a stove and a little thing to keep food in. There are suits moulded into the bubble so people can come inside but stay outside.

I was woken up the next day by Kathy, a nurse, I had to take a load of tablets - they were horrible. Then I was washed with Hibiscrub. Hibiscrub was a kind of soapy water and it made my skin go all dry. I could hardly move because my neck was hurting. All the nurses said I would get used to the tube. All the food that was passed in had to be sterilised. It took all the vitamins out of the food so I had vitamin tablets but mostly I had tablets to fight infection. The blood tests became more frequent and I only had three weeks to live but at the time I didn't know.

The next week was important because Ena was giving her bone marrow to me. Ena went down for an operation to have some out. A needle was put into my hand and two bags of blood were transplanted into my bloodstream. The marrow slowly began to work but the trouble was it was very slow.

About three weeks later the thing I had been dreading happened - my hair began to fall out due to a drug. Finally it was all gone but began growing back. I joked a lot with Jan, Susan, Aly, Cathy, Claire, Sister Meyers and all the doctors. It kept up my spirits. They checked my heart rate every day and my blood. I didn't have much to do in the bubble except play board games and cards. Me and Peter, Graham used to organise little football tournaments that was the only thing. There was another thing I liked that was wrestling with the nurses. The

physiotherapist also kept me occupied with exercises - it was hard work. After two months in the bubble I started getting into tempers. They kept telling me I would be out soon but the weeks wore on. I felt condemned by God.

"What have I done wrong!" I cried and cried. School work was getting me down. If I couldn't do it I would throw it into the bin. On some occasions I would pick up the scissors and try to kill myself.

Eventually though, my blood amount shot up and I would be coming out soon but I didn't know when. Dr. Watson phoned up Arsenal and asked them if they would come and see me. My sister Sue said that my tube would be coming out. When it did come out it was very painful and when it was out I felt like a free bird. I kept my stitches as souvenirs. The doctors meeting to decide when I would be coming out. My tempers and impatience grew more and more and I upset everybody.

Saturday morning I was excited as Arsenal were going to come and see me on Monday. The screens were pulled round for my wash and through them I could see all the nurses, relations and doctors. Suddenly I knew. My sister smiled and Dr. Watson came in and said,

"How would you like to go home to-day?"

I was so speechless all I could say was, "Okay!"

The bubble hatch was undone and I clambered out. At first it was funny and walking about I kept wobbling. I was not allowed any cake because it was from a bakery and not sterilised. My mum and dad seized me and hugged me tight. All the cameras flashed and lit up the place. I was crying, so were my mum and dad. I thought of Peter not being here - it was a pity. After the celebrations we went down to the car, the first fresh air for three months. The car was all decorated in ribbons and writing. It was great walking about. At last I was like everybody else. When we got home the dog did not recognise me and then I looked at the door and it had

"Welcome home Andrew!" on it.

I was very happy. I was home, and my dream had come true.

Transcript of unsolicited school essay by patient A.U. with acquired severe aplastic anaemia. He remains well with donor karyotype in his marrow 28 months after grafting.

## Abbreviations

| | |
|---|---|
| AA | Aplastic anaemia |
| AFRACO | Amphotericin, framycetin and colistin |
| AL | Acute leukaemia |
| ALG | Anti-lymphocyte globulin |
| ALL | Acute lymphoblastic leukaemia |
| AML | Acute myeloid leukaemia |
| ANLL | Acute non-lymphoblastic leukaemia |
| B lymphocyte | "Bursa equivalent" derived lymphocyte |
| BK | Strain of polyoma virus |
| BMT | Bone marrow transplant |
| BN | Barrier nursing cubicle |
| BNNP | Bacitracin, neomycin, nystatin and polymixin |
| $C_3$,$C_4$,C1q | Components of the complement system |
| cfm | Cubic feet per minute |
| CFU | Colony forming units |
| CFT | Complement fixation test |
| CGL | Chronic granulocytic leukaemia |
| CMV | Cytomegalovirus |
| Con-A | Convanavalin-A |
| cpm | Counts per minute |
| cps | Cycles per second |
| CSA | Cyclosporin-A |
| CY | Cyclophosphamide |
| dbA | Decibel scale A |
| DHS | Delayed hypersensitivity |
| DNCB | Dinitrochlorobenzene |
| ECG | Electrocardiogram |
| EBMT | European Bone Marrow Transplant Association |
| FA | Fanconi's anaemia |
| FRACON | Framycetin, colistin and nystatin |
| GVHD | Graft-versus-host disease |
| GVN | Gentamicin, vancomycin and nystatin |
| H2 | A mouse histocompatibility locus |
| HEPA | High efficiency particle arrester |
| HLA | Human leukocyte antigen |
| HSV | Herpes simplex virus |

| | |
|---|---|
| IDU | Idoxuridine |
| Ig (G) | Immunoglobulin (G) |
| JC | Strain of polyoma virus |
| KLH | Keyhole limpet haemocyanin |
| LAF | Laminar air flow |
| MHC | Major histocompatibility loci |
| MLC | Mixed lymphocyte culture |
| NA | Not available |
| ND | Not detected |
| NEOCON | Neomycin, colistin and nystatin |
| NHI | Normal human immunoglobulin |
| NR | Normal range |
| OPCS | Office of Population, Census and Survey |
| PA | Prophylactic antimicrobial agents |
| PE | Protected environment |
| PEPA | Combination of PE and PA |
| PHA | Phytohaemagglutinin |
| PML | Progressive multifocal leukoencephalopathy |
| PPD | Purified protein derivative |
| PPVF | Polymyxin, paromomycin, vancomycin and antifungal agents |
| PVC | Polyvinyl chloride |
| RI | Remission induction |
| RMH | Royal Marsden Hospital |
| RRI | Relative response index |
| SCID | Severe combined immune deficiency |
| SK-SD | Streptokinase, streptodornase |
| SRBCR | Sheep red blood cell rosettes |
| T lymphocyte | Thymus-derived lymphocyte |
| TBI | Total body irradiation |
| TSN | Trimethoprim, sulphamethoxazole and nystatin |
| VIG | Varicella immune globulin |
| VTI | Vickers-Trexler isolator |
| VZ | Varicella zoster |
| WCH | Westminster Children's Hospital |

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# Evaluation of Vickers-Trexler isolator in children undergoing bone marrow transplantation

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SUMMARY Four children, 5 months to 15 years of age, underwent bone marrow transplantation in Vickers-Trexler isolator tents. Two grafts were elective. During 170 days of isolation no clinical infections due to exogenous micro-organisms developed despite severe immunodeficiency. The decontamination regimen and sterile procedures used, as well as the microbiological results, are described. This form of isolation in paediatric practice was found to be highly acceptable to both patients and staff.

Isolators have been used for many years to maintain 'germ-free' or gnotobiotic animal colonies (Gordon and Pesti, 1971) and more recently in the protection of adult leukaemia patients undergoing intensive treatment (Trexler *et al.*, 1975). They have also been of value in the provision of a sterile environment for newborn babies with suspected immunological deficiency. We have used isolators for bone marrow transplantation in 4 children who were extremely susceptible to infection either due to the nature of their disease or as a result of pregraft immunosuppression. Two children had severe combined immune deficiency disease (SCID), one had chronic granulomatous disease (CGD), and one had Fanconi's hypoplastic anaemia. The marrow grafting procedures used have been described previously (Humble and Barrett, 1975).

The susceptibility to infection in these conditions depends on the degree to which cellular and humoral immunity are impaired. For example, polymorphonuclear leucocytes and monocytes in patients with CGD have greatly impaired ability to kill *Staphylococcus aureus*, *Serratia*, *Aerobacter*, and *Candida* species, and thus predispose to infection with these organisms (Oh *et al.*, 1969; Rodey *et al.*, 1969). Previous therapy and the duration of the disease or its complications will also markedly influence the bacterial flora present. Even after marrow transplantation there is an increased susceptibility to infection during the first 3 weeks due to granulocytopenia and for at least the first 3 months because of immunoincompetence (Johnson *et al.*, 1976).

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**Isolation and decontamination procedures**

In association with Mr. P. C. Trexler of the Royal Veterinary College (University of London) we have adapted isolators for paediatric use. The basic structure and management of the bed isolator (Fig. 1), including the supply of positive pressure sterile air using a 1415 l/min blower and high efficiency particle arrester (HEPA) filters, is very much as described previously (Trexler *et al.*, 1975). The infant isolator (Fig. 2) is made of the same transparent polyvinyl chloride (0·1–0·2 mm thickness). It consists of a sausage, 137×91 cm, which has four sleeves down each side—instead of half suits—and additional facilities for weighing and taking *x*-rays. The storage isolator and its manner of connection to both infant and bed versions are very similar. The infant isolator can be stored complete and sterile and is ready for use within 24 hours. The larger version is ready for use in 48 hours. Both isolators can be erected over standard hospital cots or beds in a normal ward and removed for storage when not required.

The pregraft decontamination regimens adopted were similar in all cases. The patients were initially nursed under reverse barrier conditions in a cubicle. All persons entering the cubicle wore hats, masks, gowns, gloves, and overshoes. All laundry was sterile. Good quality prepacked food was used and samples tested were found to be sterile on all occasions. All surfaces in the cubicle, including equipment, were disinfected with 1·5% Hycolin in spirit. The patients had daily baths after applying 4% chlorhexidine in detergent (Hibiscrub) to the skin; they also received thrice daily sprays of chlor-

Fig. 1 *Large isolator seen in a normal paediatric ward.*

hexidine (0·02% aqueous solution) to the nose, throat, ears, and foreskin and, on alternate days, the hair was washed with Savlon. Chlorhexidine obstetric cream was applied nightly to the vagina. Corsodyl (chlorhexidine) dental gel was applied to the gums and Naseptin to the nose twice daily.

Bowel decontamination in the nonelective cases was started in the cubicle, while in the elective cases it began in the isolator. Each patient received a combination of oral antibiotics according to the 'Fracon' regimen devised by Trexler *et al.* (1975). Administration of nystatin was started 4 days before framycetin and colistin and the doses of all antibiotics were modified according to age. Two items were changed due to patient preference. As a result of our experience with previous cases, Case 2 (Table) received proportionately lower doses of colistin to lessen the risk of gastric intolerance. The full decontamination regimen was continued until 72 hours before the planned removal of the patient from the isolator.

Once the microbial population at the various sites had reached minimal levels, the patient entered the isolator wearing only a sterile gown, which was shed on entry. Decontamination of the total body surface, bowel, and orifices was continued using sterile solutions and sterile drugs. All articles entering the isolator were sterilized as appropriate, by heat, gamma irradiation, or chemical means (Milton hypochlorite 1 in 80). Food was sterilized by gamma irradiation in commerically prepared packs. Samples for bacteriological culture were taken thrice weekly from nose, throat, mouth, urine, and vagina. Axillae, groins, hairline, ears, and any skin spots were sampled twice-weekly using swabs moistened with water for dry areas. Swabs from the tent were examined regularly. All specimens were cultured aerobically and anaerobically. The delay between sampling and culturing specimens rarely exceeded 2 hours.

In both the elective grafts a transit isolator was used after a 'sterile' bowel had been achieved. The patients were transferred to a small transit isolator for 12 hours while the original isolator was emptied, resterilized, and restocked. Case 1, because of his hyperplastic marrow, received radiotherapy to areas of the iliac crests to create a 'space' for the new marrow. For this he was transferred by van to an associated hospital in the transit isolator and, on return, re-entered the tent, strict isolation having been maintained throughout. The 2 patients with SCID (Cases 3 and 4) did not receive immunosuppressive therapy before bone marrow grafting.

## Results

During 15 months we have had 242 days of experience in treating patients—including one adult —in the two sizes of isolator. The Table summarizes clinical data, including the occurrence of leucopenia

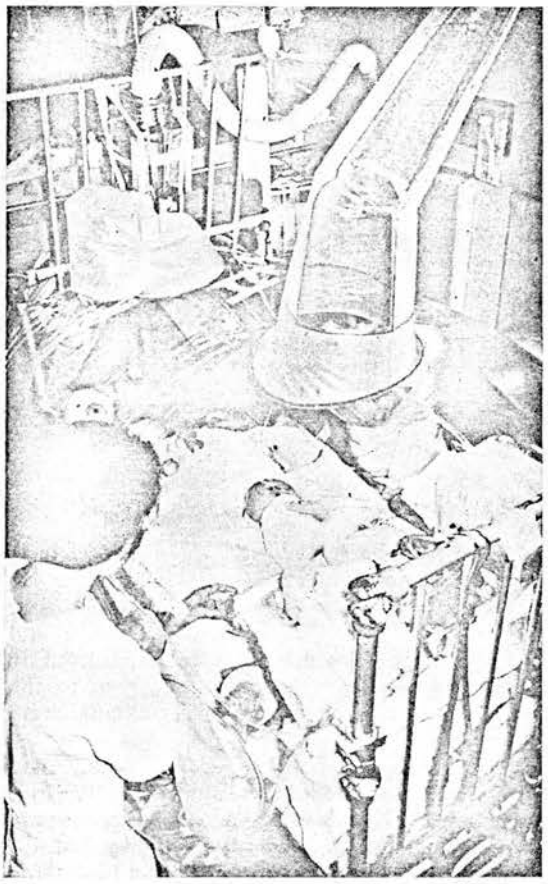

Fig. 2 *General view of cot isolator in use.*

and fever in the 4 children. The elective transplants (Cases 1, 2) were approached cautiously in view of the high risks associated with vigorous immunosuppression. In Case 1, despite routine Hibiscrub and Naseptin at home, swabs from the nose and a sternal sinus yielded *Staph. aureus* in large numbers. In addition, he had aspergillosis of the spine and had been pyrexial for several months before admission. CGD results in a hyperplastic bone marrow and the immunosuppression used while in the isolator reduced the neutrophil count to $<0{\cdot}5\times10^9$/l (500/mm$^3$) for 6 days. On 2 of these days the neutrophil count was $<0{\cdot}1\times10^9$/l with a total white cell count of $0{\cdot}5\times10^9$/l (500/mm$^3$). Using the general measures outlined above, combined with clotrimazole and amphotericin B for the aspergillosis, no further infection occurred. However, *Candida albicans* appeared in the stools 10 days after the disappearance of bacteria. He remained in the tent for a total of 31 days.

Case 2 received a graft because of his increasing blood transfusion requirements, spontaneous bruising, and infective episodes. He had no unusual organisms on entry into the tent. His stools became sterile 4 days after full implementation of the decontamination procedures, and remained microbe-free over the next 62 days. Though he was co-operative and capable of washing himself, decontamination was more effective when the nurses washed him. His white cell count stayed below $0{\cdot}5\times10^9$/l (500/mm$^3$) for 14 days and his neutrophil count below $0{\cdot}1\times10^9$/l (100/mm$^3$) for 10 days and $<0{\cdot}5\times10^9$/l (500/mm$^3$) for a further 11 days. Throughout his 72 days of isolation he remained free of infection.

The two infants (Cases 3,4), unlike the older children, required no immunosuppressive therapy and consequently neutropenia did not occur. The one patient (Case 3) who died of infection was heavily colonized by pathogens on admission. *Pseudomonas aeruginosa* was isolated from blood cultures and several other sites, and *Salmonella typhimurium* was isolated from stool cultures. Despite the decontamination regimen and ampicillin, carbenicillin, and gentamicin therapy the stools and blood cultures continued to yield salmonella and pseudomonas. A high fever, evident throughout the

Table *Summary of clinical and haematological data on patients while in isolator*

| *Case no.* | *Age (yrs) and sex* | *Diagnosis* | *No. of days in isolator* | *Duration (days) of* | | *No. of days with fever (>37 °C)* | *Superadded clinical infections‡* | *Clinical outcome* |
|---|---|---|---|---|---|---|---|---|
| | | | | *Lymphopenia** | *Neutropenia†* | | | |
| 1 | 7, M | CGD | 31 | 9 | 6 | 26 | — | Survived, with evidence of graft function for 3 m |
| 2 | 15, M | Fanconi's anaemia | 72 | 25 | 21 | | — | Survived; haematological function remains normal 15 months after graft |
| 3 | 5/12, F | SCID | 14 | 10 | — | 14 | — | Died due to pseudomonas pneumonia and septicaemia |
| 4 | 6/12, F | SCID | 53 | 13 | — | 5 | Lobar pneumonia | Died due to graft-versus-host disease |

*Lymphocytes $<0{\cdot}5\times10^9$/l; †neutrophils $<0{\cdot}5\times10^9$/l; ‡infections arising after entry into the isolator.
CGD = chronic granulomatous disease; SCID = severe combined immune deficiency disease.

interval between admission and entry into the isolator, persisted. She died 14 days after isolation due to pseudomonas pneumonia and septicaemia.

Case 4 had been in the isolator for a total of 53 days during which time small numbers of *Strep. pneumoniae* and viridans streptococci were isolated from the oropharynx, and *Staph. epidermidis* from the skin, mouth, and vagina. All of these were present before entry. The second isolation of *Strep. pneumoniae* on the 13th day after entry into the tent coincided with the development of lobar pneumonia. Penicillin therapy led to rapid resolution of the pneumonia, and the pneumococcus was eliminated.

Case 4 died of myocarditis due to graft-versus-host reaction 36 days after marrow transplantation. There was good evidence of immunological reconstitution. No viruses were identified by electron microscopy or isolated by culture, either before or after death and antibody studies were unhelpful.

In 3 of the patients, sterile stool cultures were achieved by the end of the first week of bowel decontamination. Subsequent stool cultures from these patients while in the isolator yielded no growth of organisms in 76% of the specimens. Stool cultures from the fourth patient, as already stated, continued to grow the pseudomonas and salmonella organisms present on admission.

The proportion of sterile cultures from the various other sites were as follows: axillae 92%, hairline 85%, groins 59%, ears 68%, nose 50%, throat 71%, mouth 50%, urine 87%, and vagina 91%. The majority of the positive cultures consisted of *Staph. epidermidis* while scanty faecal organisms occurred in one-fifth of the specimens.

## Discussion

Although tedious for patients and staff alike, strict protective isolation is a necessity in children with severe immunosuppression. Infection occurring in these patients after transplantation could have put impossible demands for granulocytes and platelets on the leucopheresis unit in addition to increasing the severity of any graft-versus-host disease (Thomas *et al.*, 1975).

The acceptability of the Trexler form of isolator is not in doubt. The 2 children who were old enough to be fully aware of their surroundings accepted their terms of 31 and 72 days' isolation well. Case 1 regarded the isolator as a castle to keep germs at bay. Despite numerous games, school work, and even a ciné camera inside the isolator, boredom was a major problem. This was less so with Case 2 whose isolator was placed in an open ward and he was very much more part of the ward activities. It was sometimes difficult to provide a sufficiently varied sterile diet but a small refrigerator in the supply compartment of the isolator provided useful cold drinks. Just how well the 2 infants accepted isolation is more difficult to assess. Case 4, judging by normal responses to the approach of staff and normal developmental progress while in the isolator, showed neither sensory nor emotional deprivation. Tests of vision and hearing while in the isolator gave normal results.

Parents also found the isolator acceptable in contrast to strict room isolation where they must either carry out rigorous aseptic rituals or be denied physical contact and close proximity. Though the plastic film did provide a physical barrier, parents were aware that they looked like parents to their child as they did not have to wear masks and hats. They also realized that the plastic barrier was not really different from the barrier of a gown as regards transmission of body warmth and contour, and that handling the child, whether in an isolator or in a cubicle, would require them to wear gloves. The parents were taught the isolator routines and helped considerably. They felt it easy to come and go, and for other relatives to do the same, unhindered by elaborate procedures. Communication through the plastic was very easy and no special aids were required.

For members of staff the isolator is regarded almost unanimously as a boon. Entry into a half-suit takes about 30 seconds and less than 15 seconds are needed to apply a pair of glove sleeves. Against this must be set the time taken to put on sterile clothing and the cost of numerous sets of these items. Some procedures do require novel adaptation, for example, auscultation with a stethoscope head passed down the sleeve into the gloved hand, but most procedures can be carried out normally (Fig. 3). The benefits of being able to approach and talk to the patient without formalities, such as gowning, are great, and allow much more contact between patient and staff than is usually possible during isolation.

With regard to prolonged isolation, the tent is greatly preferred to a cubicle by our nurses because, despite the need for some additional planning, the continuous maintenance of asepsis is easier and more certain.

Strict protective isolation has been shown to be superior to conventional room isolation in reducing the incidence of severe infections among adult leukaemia patients who were receiving intensive chemotherapy (Levine *et al.*, 1973, 1975). Dooren and his colleagues (de Koning *et al.*, 1969; Vossen *et al.*, 1973) have used a laminar flow system for isolation of patients undergoing bone marrow transplantation. They achieved good results in

Fig. 3 *Manipulation of nasogastric feed through glove sleeves in cot isolator.*

preventing cross-infection over long periods of time. However, this form of isolation fails to provide a complete physical barrier between the patient and his environment and 2 of the 5 Leiden patients were colonized by exogenous micro-organisms.

In a recent comprehensive review (Thomas *et al.*, 1975), infection is reported to be the 'usual proximate cause of death' during and after transplantation. The authors report that almost all of their marrow transplants were carried out using 'simple mask reverse isolation'. In an earlier series (Solberg *et al.*, 1971), 41 infections occurred in 11 patients after transplantation. Infection with exogenous organisms was encountered most frequently in patients occupying conventional isolation rooms.

Except for the persistence of pathogenic bacteria in one patient, the decontamination procedures successfully suppressed to minimal levels the pre-existing bacterial populations at the various sites. Recording, as we do, the most scanty cultures of commensal organisms tends to create a false impression of inadequacy in the decontamination procedures. However, we recognize the impossibility of maintaining true sterility of body surfaces that have previously been heavily colonized by commensals or potential pathogens. This is in contrast to the situation where babies are delivered aseptically and then maintained in completely germ-free conditions. But a full 'inventory' of even the most minimal numbers of organisms is necessary to forewarn against the hazard of subsequent overgrowth by such organisms.

The antibiotic combination used effectively suppressed the intestinal flora despite the fact that none of these agents individually is usually regarded as being active against the predominant *Bacteroides* group and other anaerobes of the bowel. This may be explained partly by the fact that these drugs achieve very high concentrations in the bowel from which they are not absorbed systemically and, in addition, an unsuitable milieu for anaerobes may result once the aerobic flora has been eradicated.

At the end of the period of decontamination and isolation patients can be discharged immediately from hospital to minimize the risks of recolonization by hospital pathogens. The alternative course which we have followed is to recolonize (or 'reconventionalize') the bowel with the donor's mixed flora after screening it for suitability.

An adult patient who recently underwent marrow transplantation at our hospital remained free of exogenous infection during 72 days' stay in the bed isolator despite a total white cell count of $<0{\cdot}1\times10^9$/l (100/mm$^3$) for 6 weeks. Throughout this time *C. albicans* was isolated from several mucosal sites and persisted despite systemic antifungal therapy. The patient eventually died of systemic candidiasis. This, and our additional experience with Case 1, has prompted us to administer nystatin or amphotericin B 4 days before colistin and framycetin in the decontamination of subsequent patients.

Because of its high acceptability from a microbiological, staff, and patient point of view, we recommend that this form of isolation be used more widely and thus undergo further evaluation.

We are grateful to Dr. K. Hugh-Jones for allowing us to report on his patients; to the nursing staff for their co-operation; to the technical staff of the Department of Bacteriology; and to the Andrew Bostic Fund for financial assistance.

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from BONE MARROW TRANSPLANTATION IN EUROPE
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# PROGRESS WITH TENT ISOLATORS

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A description of the way in which we care for transplant patients in a modified Vickers-Trexler isolator.

The flexible film isolators are situated in general paediatric wards and the patients are cared for by medical and nursing staff who have responsibility for other general paediatric patients. The isolator canopy is hung from light metal poles and the canopy kept at an automatic positive pressure using 1415 L/min blower. The ingoing air is passed through 0.3 μ HEPA filters. The bed is outside the canopy but all bedding, medical and nursing equipment is inside. Access to the patient is via built-in flexible plastic half-suits with clear visors to give an undistorted view. Each half-suit has an additional air supply to aid the person working therein. Different sizes of isolator allow appropriate care for the infant, the toddler and the older child and also allow aseptic transport of the patient to other hospital departments.

The isolator is sterilised with peracetic acid initially and thereafter anything entering the isolator must be sterilised, either by gamma radiation to 2.5 Megarads, steam autoclaving or hypochlorite disinfection. There is an entry port through which items can enter the isolator aseptically against a positive pressure air flow, and an exit port, closed by a bag, through which rubbish may be removed, the technique allowing isolation never to be broken.

Frozen food is sterilised by gamma radiation or else good quality tinned food is very carefully surface disinfected. There is a choice of 23 main dishes and 16 desserts. A cooker, kettle, toaster and a refrigerator sleeve inside the isolator allow a wide variety of foods. Most children receive total parenteral feeding for some time but this is outside the isolator and directed through a plastic cone into a central venous line.

Procedures such as lumbar puncture, electrocardiography, electroencephalography, ultrasound studies and even a barium meal have all been carried out inside the isolator.

No electronic communication aids are required and other children in the ward can play games with the tent occupant. Glove sleeves, if reversed, are used by the child to adjust television or radio sets. A telephone sleeve is provided. Parents are actively encouraged to help with

caring for their child.

There is no doubt that the nurses and medical staff feel less isolated from the patient with this form of isolation compared with cubicle isolation and to have the isolator in a ward with other children is helpful to both the isolated patient, his parents and the attending staff.

Over 782 days of exclusion isolator experience with 12 patients, including 487 days with neutrophils less than 500/cu mm and 270 days with neutrophils less than 100/cu mm, there has been only one episode of bacterial contamination which was due to a tear in the visor of a half-suit. Although patients have contracted bacterial infection whilst in the isolator these have, with this one exception, been due to organisms which were known to have entered the isolator tent with the patient despite extensive decontamination.

We have recently used an infant isolator to contain a patient with severe salmonellosis who was a danger to other patients. To convert the isolator from exclusion mode to containment took about 3 hours and the nurses found no difficulty in readapting their techniques.

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# Vickers – Trexler Isolators in Childhood Bone Marrow Transplantation

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## Vickers-Trexler Isolators in Childhood Bone Marrow Transplantation

We have used Vickers-Trexler flexible film isolators for thirteen bone marrow transplant children in the past 3 years. Prior to isolator entry there is an extensive surface and orifice decontamination regime based mainly on Chlorhexidine preparations. Oral amphotericin, started 4 days before colomycin and framycetin is used to decontaminate the gut. These regimes are continued in the isolator where all equipment and almost all food and drugs have been sterilised with either 2.5 Megarads of gamma radiation, steam autoclave or filtered through 0.22 μmillepore filters.

The bed isolators are very similar to those already described[1] A modification to the mattress pocket of the patient envelope allows a cot to be placed within the isolator. This is used for toddlers. A smaller isolator with glove sleeves instead of half suits is used for infants and a slightly larger version is used as a transit isolator to take decontaminated patients to other hospital departments without breaking sterility. Any hardware is compatible with all the isolators.

We have introduced a number of modifications to the original envelope design. Many more glove sleeves give better access to all parts of the isolator and additional cones allow permanent leads for electrocardiology, electroencephalography, oxygen supply (filtered) and concentration monitoring and a ripple mattress. Intravenous feeding is given from outside the isolator through a cone with the tubing being changed everyday. A full electricity supply is available inside, controlled from an external plug board. A sleeve continuous with the envelope fits inside a refrigerator so sterile drinks and drugs can be kept cold if required. Each half suit has four arms allowing doctors and nurses to use different sizes of gloves. A large pocket and long sleeve allows many types of x-rays to be taken including a barium meal.

On one occasion we have converted our exclusion isolator to the containment mode and successfully prevented any cross-infection in a general ward when nursing a baby with severe salmonellosis over 120 days. The conversion took about 5 hours and no difficulties were experienced by the nurses who were used to exclusion mode nursing.

Air sampling studies confirm the efficiency of the isolator in the general ward, compared to the ward and a reverse barrier nursing cubicle.

Thirteen transplant children have now spent 823 days in the isolators and on only one occasion has an organism been known to have gained access to the isolator. This was through a tear in half suit visor, the design of which is now different. These 823 days of isolation include 535 days with a granulocyte count of less than 500/cu mm and 285 days with less than 100 cu/mm. Most children have become infected in the isolator but with the exception above, it has always been with an organism known to have entered with the patient or caused septicaemia previously in the patient.

We firmly believe that the isolator tent system provides a practical and reliable method of nursing the severely immunocomprised patient within a general paediatric ward. The capital

costs are low; the space used is very flexible and the nurses much prefer the isolator to the less reliable aseptic rituals of the cubicle.

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# Letters to the Editor

## ANTIBIOTIC PROPHYLAXIS FOR PATIENTS IN PROTECTIVE ISOLATION

SIR,—In October, 1977, you published a paper[1] from the Royal Marsden, Hammersmith, and St. Mary's Hospitals on patients with acute non-lymphoblastic leukæmia nursed in protective isolation and given the oral non-absorbable antibiotics framycetin, colistin, and nystatin (FRACON), together with cutaneous and mucosal antiseptic preparations (mainly chlorhexidine compounds), or none of these. The patients on antibiotic prophylaxis had significantly fewer infections, fewer deaths from infection, fewer pyrexial episodes, and less systemic antibiotic therapy than did the patients who, though similarly nursed in protective isolation, did not receive this regimen.

Since the beginning of 1978 the regimen originally described has been altered to halve the number of tablets taken and to reduce greatly the topical antiseptics, whilst maintaining adequate suppression of microbial flora. Oral neomycin has replaced framycetin, thereby halving the cost of each dose. The only topical antiseptic used routinely is twice daily chlorhexidine obstetric cream (1%) to the vagina and vulva. Chlorhexidine mouthwashes (0·02% aqueous) are used only if necessary.

From Jan. 1, 1978, to April 1, 1979, this new regimen has been used on 38 occasions, all patients having acute non-lymphoblastic leukæmia, newly diagnosed or in first relapse. The comparison between the previous regimen and the neomycin, colistin, nystatin (NEOCON) regimen is shown in the accompanying table. Patients intolerant of the regimen were not included in these studies. Despite chemotherapy policies being if anything more anti-leukæmic, it would seem that there is slightly less severe neutropenia, although the time with less than 500 neutrophils/μl is the same. The differences in fever days and days of systemic antibiotic therapy are insignificant, and no patient has died of infection in the present series.

COMPARISON OF FRACON AND NEOCON REGIMENS

| — | FRACON | NEOCON |
|---|---|---|
| Patients intolerant of regimen | 5 | 4 |
| Patients studied | 46 | 34 |
| Total patient-days on study | 1790 | 1138 |
| Days pyrexial (>38°C) | 315 *(18%)* | 161 *(14%)* |
| Days on systemic antibiotics | 808 *(45%)* | 531 *(47%)* |
| % of study days with neutrophil-count | | |
| <100/μl | 41 | 28 |
| 101–500/μl | 32 | 40 |
| 501–1000/μl | 11 | 12 |

Stools were judged satisfactory in 91% of patients studied. 9 patients had unsatisfactory stools. 1 patient's stools were considered unsatisfactory since pseudomonas was grown on one occasion from the stool and the other two were unsatisfactory in that *Escherichia coli* could not be eliminated. 4 patients were excluded from the study as they could not tolerate even this reduced amount of gut antibiotics.

In addition, 14 acute non-lymphoblastic leukæmic patients who tolerated the NEOCON regimen underwent marrow transplantation with cyclophosphamide and total body irradiation. These patients received the same regimen and protective isolation for 384 days during which time they had 55 (14%) days of pyrexia and 139 (36%) days of systemic antibiotics. There is no evidence that oral cyclosporin A given as prophylaxis against graft-versus-host disease makes any difference to the stool bacterial flora.

On the grounds of patient comfort and economy the Royal Marsden Hospital leukæmia unit now suggests that the following regimen along with protective isolation gives practical decontamination for patients with acute non-lymphoblastic leukæmia:

| | |
|---|---|
| Neomycin sulphate 500 mg | one tablet twice daily |
| Colistin sulphate $1·5 \times 10^6$ units | one tablet twice daily |
| Nystatin $0·5 \times 10^6$ units, | one tablet twice daily |
| Nystatin $0·1 \times 10^6$ units, | as syrup twice daily |
| Chlorhexidine obstetric cream (1%) twice daily | to vagina and vulva |
| Amphotericin B lozenges (10 mg) | sucked four times a day |

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[1] Storring, R. A., Jameson, B., McElwain, T. J., Wiltshaw, E., Spiers, A. S. D., Gaya, H. *Lancet*, 1977, ii, 837.

# CLINICAL RESULTS OF BONE MARROW TRANSPLANTATION IN SCID AND OTHER IMMUNODEFICIENCY STATES

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The five infants diagnosed as SCID conformed to the original Swiss-type description, all having severe lymphopenia (180-600/mm$^3$) and virtually absent SRBC rosettes, PHA, *Candida* and MLR responses. Three of the 5 had lymphocytes behaving as poor stimulating cells in the MLR, which makes matching difficult. ADA, NP and transcobalamin deficiencies were excluded in all 5. All 5 also showed absence of Iga, with normal free secretory piece in their saliva, and <2 per cent of peripheral blood lymphocytes bearing Ig. Four were carefully examined with reliable fluorescent anti-IgD and none was detected. IgM levels before plasma therapy were all below 9 mg/dl and isohaemagglutinins were all absent. Patient 3 did however show an IgE level of 350 iu/ml, with 80 IgE-bearing cells/mm$^3$, supporting a hypothesis[1] that IgE is the most primitive of the immunoglobulins.

GVHD occurred in patients 3 and 5 as predicted by the SI's; donor vs Recipient of 1.83 and 3.5 respectively (>1.6 criterion ascertained using a well standardised MLR[2]). In patient 3 this was anticipated and modified by a single dose (8 mg/kg) of horse ATG on day 7 between methotrexate on days 6 and 8. This appeared to abort the acute GVHD, but a chronic obstructive jaundice with soft xanthomatosis did follow, cleared by oral choestyramine therapy. The T-cell reconstitution was delayed till 9/12 post-graft, with an overshoot of the B-cells presumably due to lack of T-suppressor-cells. This patient is reported elsewhere (3,4).

The other long-term survivor, patient 1, is reported elsewhere (5). In the two early deaths (patients 2, 4) post-mortem microscopy showed early population of otherwise empty lymphoid tissues by mature-looking immuno-blasts, some containing IgM and IgG. It was also interesting to observe that IIIs secretory IgA appeared early in saliva in the other 3 patients (yet obviously derived from bone marrow precursors that normally release 7S IgA (6) and serum IgA was the last immunoglobulin to appear, up to one year post-graft.

Patients 6-10 all had good MLR responses (indeed, 7 and 10 had results above our normal range) so we believed there was less risk of GVHD, and indeed none suffered that complication. Because of their severe symptomatic immune deficiency, we were loth to suppress their compensating T-cells, and only prepared patient 7 and 10 who were to receive unrelated marrow.

**Westminster hospitals bone marrow transplant team.**
**Summary of bone marrow transplant data for S.C.I.D. as at 10 march 1977.**

| Diagnosis | Number of transfusions Blood Random | Platelets Random | Platelets Family | Refractory to random platelets | Kind of antibodies present in recipient | Number of pregnancies | Sex/âge (yr) Rec | Sex/âge (yr) Don | HL-A Rec | HL-A Don | ABO R | ABO D | S.I. D→R / R→D |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S.C.I.D. | None | None | None | No | None | None | F 7/12 | M4 | 2,3 ; 7,13 | Identical sibling | 0 | 0 | 0.6 / < 1 |
| S.C.I.D. | None | None | None | No | None | None | M 6/12 | M7 | 1,9 ; 4,8 c | Identical sibling | 0 | 0 | 1.0 / < 1 |
| S.C.I.D. | Non | None | None | No | Non | None | M 5/12 | M24 | 1,9 ; 8,12 | Identical Father | A Pos. | A Neg. | 1.8 / < 1 |
| S.C.I.D. | None | None | None | No | None | None | F 8/12 | F32 | 1,15 W ; 3,12 | 1,17 ; 3,12 Aunt | A Neg. | A Pos. | 1.6 / < 1 |
| S.C.I.D. | None | None | None | No | None | None | F 5/12 | F13 | 2,9 ; 12,15 W | 2 - ; 14 - Aunt | A | 0 | 3.5 / < 1 |

**Westminster hospitals bone marrow transplant team.**
**Summary of bone marrow transplant data for SCID as at 10 th march 1977.**

| Preparation before grafting | Marrow celles infused (x $10^8$/kg) | Engraftment (day) Method of investigation | Chimerism (C) = complete (M) = mixed | Autologous recovery ? | Graft rejection (day) | Clinical GvHD (grade) (*) treated with ATG | Survival (days) | Clinical course or cause of death |
|---|---|---|---|---|---|---|---|---|
| Nil | 0.7 | Yes (27) Retics 20 % Lympho 20,000 PHA POS | (M) ♦ (C) 75 % PHA at 3/12 ♦ 100 % at 1 ½ yr | B-cell sex not known | No | None | 2 069 | Alive and well Full immunocompetence |
| Nil | 5.5 | Yes (8 histology+) | M (chromosomes) | Not evaluable | No | Not up to day 8 | 8 | Pre-existing stress — Curling's ulcer and haemorrhage |
| Nil | 3.0 | Yes (6) GVHD | Probably (C) as now Rh. neg. | No B-cell markers to evaluate | No | Grade III (*) | 1 539 | Fully immunocompetent Alive and well |
| Nil | 0.7 | Yes (14, histology+) | M (chromosomes) | Not evaluable | No | Not up to day 14 | 14 | Pre-existing infection : Ps. pyocyaneus septicaemia |
| Nil | 11 | Yes (6) GVHD | Not evaluable | Not evaluable | No | Grade III (*) only on day 36 | 37 | Heart block due to GVHD |
| | | + Immunoblasts by microscopy | | | | | | |

**Westminster hospitals bone marrow transplant team.**
**Summary of bone marrow transplant data for other immunodeficiency states as at 10.3.77.**

| Diagnosis | Number of transfusions Blood | Platelets Random | Platelets Random Family | Refractory to random platelets | Kind of antibodies present in recipient | Number of pregnancies | Sex/Age (yr) Rec | Don | HL-A Rec | HL-A Don | ABO R | ABO D | S.I. D ♦ R / R ♦ D |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chronic mucocutaneous Candidiasis total MIF deficiency | None | None | None | No | No blocking antibodies detected | None | M12 | M4 | 2, W19, W12 | Identical Brother | 0 | 0 | 0.9 / 1.0 |
| Chronic Granulomatous disease | None | None | None | No | High levels IgG, IgA, IgM | None | M 20/12 | F26 | 1,7 ; 1,8 | Identical Unrelated | 0 | 0 | 1.1 / 1.1 |
| Sporadic panhypo γ - globulinaemia | None | None | None | No | None | None | F9 | F17 | 11, W5 ; 2,17 | Identical Sister | A | A | 1.1 / 0.9 |
| Sporadic hanhypo γ - globulinaemia | None | None | None | No | None | None | M6 | F17 | 11, W5 ; 2,17 | Identical Sister | A | A | 0.9 / 1.6 |
| Chronic granulomatous disease | None | None | None | No | Nigh levels IgG, IgA, IgM | None | M7 | M28 | 2 - ; 8, 12 | Identical Unrelated | 0 | 0 | 1.2 / 1.0 |

**Westminster hospital bone marrow transplant team.**
**Summary of bone transplant data for other immunodeficiency states as at 10.3.77**

| Preparation before grafting | Marrow celles infused (x $10^8$/kg) | Engraftment (day) Method of investigation | Chimerism (C) = complete (M) = mixed | Autologous recovery ? (day) | Graft rejection (day) | Clinical GvHD (grade) | Survival (days) | Clinical course ; or cause of death |
|---|---|---|---|---|---|---|---|---|
| Nil | 1.0 Separated lymphocytes | (30) Acquisition of DH and MIF production | Presumed M | Unlikely as relapsed at 6 yrs, possible life of donated lymphocytes | No | No | 2 413 | Responded again to therapy. Alive and well |
| Cy 60 mg/kg × 4 | 4.2 | (12) Female NBT + polys | M | No | No | No | 900 | Alive and well |
| Nil | 1.7 | No | — | No | Possibly (S.I. 2.1 at 3/12) | No | 714 | Died of chronic bronchiectasis |
| Nil | 1.9 | No | — | No | No | No | 1 083 | Alive and well |
| ICRF 250 mg × 3 days CY 30 mg/kg × 1 + 800 topelvis only | 3.3 | (10 - 22) 1 600/mm³ NBT + po-lys. | Transient for about 3/12 | No | Yes S.I. 11.7 post-graft | No | 774 | Temporarily much improved. Paraplegia recovered. Alive |

Patient 6 had the type I defect already described (7), with normal thymidine uptake against PHA, *Candida*, MLR and PPD but with total absence of MIF production. This had not responded to iron therapy or transfer factor and the details of the intended lymphocyte graft are already published (8).

It was clinically successful, but *Candida* lesions and MIF deficiency reappeared some six years later, unresponsive to miconazole, possibly compatible with the expected life of committed donor cells. MLR in each direction was still compatible 1.1/1.1 but the lesions have cleared for 6/12 on levamisole treatment, which could have stimulated marginal residual immunity.

Patient 7 is reported elsewhere (9), and patient 10 showed marked clinical improvement before his graft diminished at 3/12 with the appearance of a positive MLR. He had a paraplegia due to spinal aspergillosis, which showed marked improvement so he is now walking normally.

Patients 8 and 9 were not doing well on gammaglobulin injections and showed marked clinical improvement for 6/12 post-graft, with reduction of bronchiectatic sputum and symptoms and of their pre-existing splenomegaly. Patient 8 slowly relapsed and eventually died of her advancing bronchiectasis. Patient 9 has been well-controlled for two years but his chest symptoms are now beginning to relapse. Studies of the interactions of T- and B-lymphocytes between donor and patients failed to show T-cell suppression of immunoglobulin synthesis, but postgraft there has been no real evidence of normal antibody production in either recipient. Evidence of graft rejection is only borderline in patient 8, with none in patient 9.

These five grafts for other immunodeficiency diseases are encouraging in that no harm was done to any of them, long-lasting good to two and worthwhile transient good to the other three.

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# CLINICAL RESULTS OF BONE MARROW TRANSPLANTATION IN APLASIA

**Report of Westminster Hospital Bone Marrow Transplant Team.**

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Our early experience with bone marrow transplantation for aplasia was very discouraging. Patients were referred late, infected and after many transfusions. We matched two patients with related donors, and another five patients with unrelated donors, finding no significant increase in well standardised (1) mixed lymphocyte reactions set up in each direction. These seven patients all died within 1-6 days of such donors having been verified and the real test of having attempted the actual graft could not be made.

We were therefore encouraged by the report of Lohrmann et *al.* (2) and the other work reported at this meeting, which suggests that an early decision can be made as to which patients have the worst prognosis. It is now clear that every transfusion increases the risks of sensitising patients with less subsequent chance of successful grafts [less than 15 = 86 % survive ; greater than 50 = 32 % survive (3)].

The first three of the six patients who underwent bone marrow transplantation were similarly transfered as emergencies, already infected. The first two patients were nursed by reverse barrier technique, and both died of infection in about one month. Patients 3-6 underwent decontamination and were managed in Vickers.

Trexler isolators, and as can be seen no gram-negative septacaemia occured. It is of interest that the unrelated grafts to patients 2 and 3 were not followed by graft-versus-host disease, nor was an unrelated graft to a seventh patient (still to be assessed) who has shown bone marrow recovery.

Patients 1 and 3 both showed acute graft-versus-host disease, even though they were transplanted from HL-A identical sisters, although this was suggested by the S.I.1.8 in Patient 3 which shows a significant difference by the method used (1). They were both treated with anti-lymphocyte globulin and indeed in both cases the graft-versus-host disease and the graft were lost. Subsequent assays (4) showed that the batches of horse ALG used were in fact, suppressor on colony forming cell units, and it is possible that early use of ALG abrogated potential grafts.

Patients 4 [reported elsewehere (5)] and 5 have both suffered extensive vital infection following full preparation and a course of ATG. The subsequent recovery of their T-cell function has been extremely slow, taking in Case 4 almost a year to come to the lower limits of normal.

Of the nine bone marrow attempted in the six patients, no less than four clearly showed no evidence of engraftment, and this emphasises the need in some way to be able to assess the soil in which the grafts are to be planted. With reliable tests, plasma factors (6) could be reduced by extensive plasma exchange or immune lymphocytes by ATG.

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# BONE-MARROW TRANSPLANT FROM AN UNRELATED DONOR FOR CHRONIC GRANULOMATOUS DISEASE

THE WESTMINSTER HOSPITALS BONE-MARROW TRANSPLANT TEAM*

***Summary*** A boy with chronic granulomatous disease received a bone-marrow graft from a fully compatible unrelated donor, in association with a modified immunosuppressant regimen. He showed considerable clinical improvement with definite evidence of engraftment, and he has enjoyed a normal life for over 3 years.

## Introduction

IN chronic granulomatous disease (C.G.D.) intracellular killing of microorganisms is impaired,[1] and invading organisms can be transported alive around the body so that "cold" granulomatous abscesses (due to the lack of heating from phagocyte activation) can form in regional lymph-nodes, liver, bones, and elsewhere. Furthermore, organisms of low-grade virulence, non-pathogenic in normal individuals, can become pathogenic. Even with

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modern antimicrobial chemotherapy, few affected children live to adulthood, and a third die before the age of 7.[2] At the present time, bone-marrow transplantation offers the only possible cure for this disease.

## Bone-marrow Transplantation

No HLA-compatible family donors were available, and volunteer unrelated donors were chosen through the Westminster Hospital Bone Marrow Donor Panel by HLA matching and subsequent mixed-lymphocyte reactions (M.L.R.).[3] The methods of donor handling and obtaining marrow have been previously described.[4] Donor cells in the host circulation were identified by a positive nitroblue-tetrazolium (N.B.T.) test and (because the donor was female) by nuclear clubs in the neutrophils. The patient was isolated with reverse-barrier nursing. This was maintained while the polymorph-count was below $2{\cdot}5\times10^9$/l. No prophylaxis for graft-versus-host reaction was given.

## Laboratory Methods

In the N.B.T. test,[5] ingestion of yeast and the actual numbers of positive (blue) cells were checked by microscopy, since this allows clear recognition of the Lyon effect in carriers of the commonest variety of C.G.D.[1] The capacity of phagocyte-rich leucocytes to kill organisms was measured by the candida-killing test.[6]

Standardised M.L.R.[3] were carried out in one direction at a time, especially post-graft, to detect any developing reaction of the recipient against the donor.

## Case-history

The patient was born in 1971, the second son of unrelated parents. The diagnosis of C.G.D. was suggested by the clinical history and confirmed by numerous zero N.B.T. scores and by candida-killing-test results of less than 6% both in his own and in normal plasma. His elder brother had also had C.G.D. and had died of Klebsiella septicæmia at the age of 2 yr. His mother has recurrent minor infections, and her stimulated N.B.T. score was 50%, confirming the Lyon effect and the X-linked inheritance of the patient's C.G.D. At 9 mo he developed a submental abscess which was drained but healed slowly. In his first 18 mo he had repeated skin sepsis and ten respiratory infections requiring antibiotics. In March, 1973, at age 20 mo, he was admitted to the Westminster Children's Hospital with a temperature of 39·5°C, septic lesions on his legs and buttocks, and severe lymphadenopathy due to exudative tonsillitis. Treatment with ampicillin and cloxacillin started before admission was continued until the initial throat-swab yielded a penicillin-sensitive group-G hæmolytic streptococcus and blood-cultures grew ampicillin-resistant *Escherichia coli*. Bacilli were identified in his peripheral-blood polymorphs. Urine and chest X-ray were normal. Treatment was changed to benzylpenicillin and chloramphenicol—a combination justifiable only in special circumstances. Within 48 h he improved and was discharged 10 days later. From age 9 to 20 mo tests revealed elevation of plasma-C3 (2·0–3·5 g/l, normal <1·7 g/l), total white-cell count (18–31 $\times10^9$/l), neutrophil-count (9–27 $\times10^9$/l), monocyte-count ($7{\cdot}6–12\times10^8$/l), serum-IgG (16–23 g/l), and serum-IgA (1·7–2·9 g/l). He showed good responses to oral poliomyelitis vaccination and tetanus-toxoid injection, and his T lymphocytes gave strong (normal) reactions against phytohæmagglutinin, candida immunogen, and foreign cells. The overall picture was of striking compensatory activity of his B and T lymphocytes. His phagocytes behaved normally in exercise and chemotaxis tests.

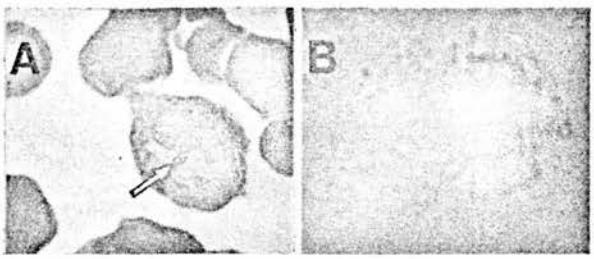
Because of his susceptibility to infection, and in view of the death of his brother from septicæmia, bone-marrow transplantation was considered. The parents fully understood the situation and the risks involved. 7 unrelated donors were found identical to the patient in ABO and rhesus blood-groups and in HLA antigens (A and B loci). Mixed-lymphocyte cultures in each direction showed 1 to be fully compatible (identical at the D locus) with the recipient (see table). In the year following the graft another 4 HLA-identical donors were found, 1 of whom was identical with the recipient at the D locus.

In April, 1973, he was admitted for marrow grafting, after further respiratory infection with otitis media and new septic skin lesions. He was given cholorhexidine baths and oral penicillin and was nursed under reverse-barrier conditions. He received 780 mg cyclophosphamide (60 mg/kg) intravenously 4 days before infusion of $5{\cdot}5\times10^9$ nucleated marrow cells from the female donor. The peripheral-leucocyte count fell to $0{\cdot}5\times10^9$/l 9 days after grafting. 12 days after the graft, occasional neutrophils with nuclear clubs (i.e., female cells) were seen and were found to be N.B.T.-positive. 42 days after the graft, 5% of the polymorphs were N.B.T.-positive, and 90 days after the graft, 12% (480/mm³) were N.B.T.-positive. At this time candida killing showed a significant rise to 13%. Since then the total leucocyte-count has remained nearly always in the normal range. Plasma-C3 and serum-Ig have also remained within normal limits. Since the graft the patient has shown striking clinical improvement, and he now goes to a normal nursery school. In over 3 yr he has had only two (largely precautionary) admissions to hospital with respiratory infections. Under reverse-barrier nursing these responded promptly to antibiotics. He has also had occasional septic spots. When he is well the stimulated N.B.T. score is around $1–5\times10^8$/l, but it increases to $4–16\times10^8$/l during a mild infection. Repeat M.L.R. between patient and donor at 4 wk, 3 mo, and 1 yr after the graft showed no evidence of sensitisation of patient to donor cells (see table).

In chromosome studies of two phytohæmagglutinin-stimulated peripheral-blood samples the female karyotype was not seen in any of the 100 mitoses examined. Before the graft <0·2% of the polymorphonuclear neutrophils (P.M.N.) showed possible "female" nuclear clubs. 12 days after the graft, characteristic clubs were found in 1·5% of the P.M.N. and this

COMPATIBILITY DATA

| Subject | Blood-groups | HLA type | Transformation indices (>1·62 is significant at the 2·5% level) | | | |
|---|---|---|---|---|---|---|
| | | | Control blocked allogeneic cells | Relation in time to graft | Recipient→blocked donor | Donor→blocked recipient |
| Recipient | O, Rh positive | 1,7,1,8 | 55·0 | Before | 1·3 | 1·1 |
| | | | | 4 wk after | 1·0 | 1·0 |
| Donor | O, Rh positive | 1,7,1,8 | 23·0 | 3 mo after | 0·9 | 1·1 |
| | | | | 1 yr after | 1·1 | 1·0 |




**After the graft, neutrophils showing female clubs (arrowed in A) were found in the blood. These cells were N.B.T-positive, unlike the recipient's neutrophils (B).**

rose at 3 mo to 2·5%. From 9 mo to 2 yr they persisted at 1·3–1·9% of the P.M.N. while the patient was well. By observing P.M.N. ingesting yeast and fixing some at the earliest stage of bluing to an N.B.T.-positive state, it has been possible to show female clubs and N.B.T. positivity in the same cells (see figure). During the third year after the graft these cells seem somewhat scantier, and it may be that the engrafted stem-cells giving rise to P.M.N. have no selective survival value in the bone-marrow itself and may finally be simply displaced by the vast majority of C.G.D. stem-cells.

However, since the patient shows no evidence of immunological rejection of the donor (or of a subsequent potential donor), it should be possible to "top up" with good stem-cells if the need arises.

## Discussion

The clinical history of these two brothers and the laboratory and family data are diagnostic of X-linked C.G.D. The severity of infections in the first boy suggested that the disease in this family had a bad prognosis.

In C.G.D., since the phagocytes cannot kill many organisms, it is important to use bactericidal antibiotics. It is also preferable to use those that can penetrate cells to kill ingested organisms, which would otherwise have a safe carriage around the body. Ezer and Soothill[7] advocate the use of rifampicin in C.G.D. Chloramphenicol is likewise useful, despite its small risk of agranulocytosis. This drug, although only bacteriostatic, penetrates cells very well, unlike the β-lactam antibiotics and aminoglycosides. It is indicated in patients with severe gram-negative infections, since septicæmia can strike very swiftly, as it did in both brothers. Antibiotics should, however, be used only in short intensive courses, to minimise the risk of resistant bacteria and fungi emerging.

In infective crises leucocytes from healthy donors and also from patients with chronic granulocytic leukæmia (C.G.L.) have been used. Since polymorphonuclear leucocytes are short-lived phagocytes, such treatment has to be repeated often during severe infection. A leucapheresis unit with a cell separator ensures adequate supplies of leucocytes. Healthy donors can, after exercise and intravenous hydrocortisone, provide white blood-cells which usually function better than those from patients with chronic myeloid leukæmia. Such donations must be irradiated with 1500r before being given to an immunosuppressed patient, to prevent any graft-versus-host tendency or acquisition of C.G.L. Repeated use of such transfusions is likely to sensitise the recipient to HLA, so they should be reserved for life-threatening crises.

Despite all these supportive measures, the patient's elder brother died before a graft could be attempted, so it was decided to proceed with a bone-marrow graft in the second child, who rapidly became infected when not on antibiotics. The objective of a bone-marrow graft is to provide a lasting source of healthy phagocytes from the stem-cells of a healthy donor. Pre-graft administration of a modest dose of cyclophosphamide to the patient was to make room for the graft in his bone-marrow, which was always more active than normal (total leucocytes 18–31 × $10^9$/l). It would also have encouraged engraftment by inhibiting, to some extent, the host's reaction, but we felt it necessary to err on the side of undersuppression because reverse-barrier nursing was our only means of preventing infection at that time. In our patients with C.G.D., other immune reactions usually show a compensatory increase, so that their serum-immunoglobulin levels are continually raised, and their M.L.R. against foreign cells are usually very high. For these reasons, the risk of a graft-versus-host reaction seems smaller than, for example, in infants with severe combined immunodeficiency.

### *Evidence of Engraftment*

Chromosome evidence has not been obtained, but Dr M. Berenbaum has calculated that the course of cyclophosphamide would have killed only 1–3% of the leucocyte precursors. This is in accord with the numbers of N.B.T.-positive polymorphs which also show nuclear clubs characteristic of female cells. Over 300 spontaneous bone-marrow mitoses would have to be examined to exclude the presence of female chromosomes, and we are not prepared to subject the patient to unnecessary marrow biopsies. Among T lymphocytes, which have a much slower turnover and are less sensitive to cyclophosphamide, female cells are probably even more difficult to detect.

It is possible that the initial clinical improvement could have been due to a "spring-clean" action of the infused normal cells, but N.B.T.-positive polymorphs could be expected to disappear within 48 h. Reutilisation of messenger R.N.A. and the "missing enzyme" could prolong N.B.T.-positivity, but this would not be expected to last 3 yr.

The evidence for engraftment therefore rests on the direct demonstration on many occasions of N.B.T.-positive polymorphs (never seen before the graft although sought many times) with the acquisition of typical female nuclear clubs and with clear failure to detect any M.L.R. against the donor on three occasions after the graft. To this can be added the clinical improvement maintained now for over 3 yr, with normalisation of the total leucocyte-count and C3 and Ig levels, all of which were persistently raised before the graft.

Certainly the procedure has been worth while. We have learned that in a crowded marrow (where abnormal phagocytes respond just as well as normal ones to chemotactic and other stimuli) it is important to "make room" for the intended graft, and this is now our practice. With the guidance of Dr K. A. Newton, it has been possible to irradiate a large part of the iliac bones without involving important growing areas. In conclusion, over 3 yr of observation have shown that unrelated bone-marrow, found by HLA typing and mixed-lymphocyte reactions, can be transplanted successfully, and that 1–5 × $10^8$/l healthy phagocytes can produce good prophylaxis against infection in chronic granulomatous

disease. We are encouraged to continue the search for unrelated donors when no matched family donor can be found.

We gratefully acknowledge the expert nursing care of this patient by Sister Lockyer and the staff of Muriel Leslie Gamage Ward, Westminster Children's Hospital. We are also indebted to the Fane Trust and the Lawson Trust, without whose help this work could never have been done. We are grateful to many colleagues in the British National Blood Transfusion Service and overseas who helped in the search for the donor.

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# STUDIES OF CYTOMEGALOVIRUS IN DONORS AND RECIPIENTS

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SUMMARY

Screening of HLA-matched donor panels for CMV antibody status has revealed groups with higher than expected numbers of antibody carriers; this is tentatively linked in this population with HLA-BW15. A case of systemic CMV infection due to transfusion following marrow transplantation is described, with details of successful treatment with adenine arabinoside.

In the course of screening several 'panels' of volunteer, HLA-matched but otherwise unrelated donors in connection with a marrow transplant programme, it was noted that 2 panels, both sharing the tissue type HLA-BW15, had higher than average numbers of individuals (9/10 and 3/4) with antibodies to CMV. On further testing of donors possessing this tissue type, CMV antibodies were found in 16 out of 22 (73%). The donor population was drawn from healthy adults in the Greater London area, and the frequency of CMV antibody for all the donors so far tested was 49%, which is comparable to the 54% reported for an age-matched population examined in London in an earlier survey (see Table 1) (1).

The 22 donors (mean age 35.3 years) with the tissue type HLA-BW15 (16 of whom were CMV antibody-positive) were compared with the 60 donors (mean age 34.1 years) possessing other tissue types (24 of whom were CMV antibody-positive), in a Fisher exact probability test, one-tailed since the direction of the difference was predicted in advance. The analysis indicated a low probability ($P = 0.009$) that the different frequencies of CMV antibody were due to chance alone.

The findings suggest that normal donors with tissue type HLA-BW15 either have greater susceptibility to CMV infection or respond more readily with antibody production than does the general population. A similar association in the mouse between CMV susceptibility and the murine major histocompatibility complex has recently been reported (2).

Thus, in some transplant cases it may be difficult to provide CMV-free donations from unrelated donor panels. Two of our recent cases with this 'susceptible' tissue type have been infected post-graft by transfusion of fresh

TABLE 1. HLA-BW15 and cytomegalovirus antibody

| | CMV antibodies | | | |
|---|---|---|---|---|
| | + | - | % positive | P* |
| HLA-BW15 (22) | 16 | 6 | 73 | <0.01 |
| Other HLA (60) | 24 | 36 | 40 | N.S. |
| All donors (82) | 40 | 42 | 49 | N.S. |
| Stern and Elek** (199) | 108 | 91 | 54 | N.S. |

*Fisher exact probability test (one-tailed).

**See References (1).

(irradiated) blood products. One was asymptomatic; the other had a severe clinical syndrome with systemic CMV infection and graft-versus-host disease. The latter case was treated successfully with adenine arabinoside (Vidarasine) and will be described in more detail.

A 15-year-old female with severe idiopathic aplastic anaemia received a bone marrow graft from her histocompatible 11-year-old sister. She was managed in strict protective isolation. Eleven days post-graft, she developed fever, diarrhoea, elevated hepatic enzymes and a haemorrhagic morbilliform rash. Skin biopsy showed evidence of graft-versus-host disease for which she was given antilymphocyte globulin. This was followed by clinical improvement but slow haematological recovery.

Forty-five days after grafting, pyrexia recurred, with mental confusion, jaundice and severe diarrhoea, but no evidence of interstitial pneumonia. There was a significant elevation of serum antibody to CMV. However, despite strong clinical suspicion of CMV infection, no chemotherapy was administered until virus was isolated from culture of her peripheral blood white cells in human embryonic lung tissue (see Fig. 1).

She was given a course of adenine arabinoside in a dose of 15 mg/kg/day which was discontinued on the 8th day due to suppression of the total leucocyte count and reticulocyte count. The chemotherapy resulted in cessation of virus growth after the last day of treatment, and subsequently the white cell count recovered to satisfactory levels. Eight months post-graft she was re-admitted to hospital with fever and mild jaundice. The CMV IgG antibody titre, which had been progressively rising, was now 1:32,000, with virus-specific IgM antibodies present. However, attempts at demonstrating the virus by electron microscopy of the urine and cultures of urine, stools, white cells and from the oropharynx proved unrewarding. She was managed conservatively and her symptoms resolved with eventual recovery.

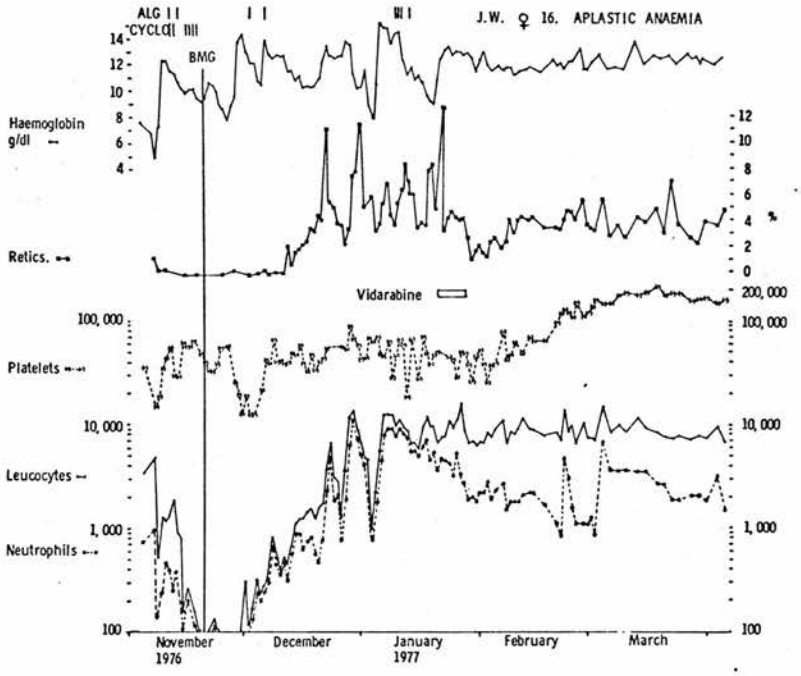


Fig. 1.

Adenine arabinoside is a relatively new antiviral agent. Although early trials have indicated that it may be effective in the treatment of human CMV infections (3), doubt has recently been cast on its efficacy in such cases (4). Its successful rise in our patient, in producing clinical recovery, suggests that it may be valuable even during the emergence of a marrow graft. Although there was undoubted myelosuppression, the graft was not ablated and recovery occurred following cessation of therapy. The recurrence of symptoms, with a markedly raised antibody titre, suggests that despite the chemotherapy, virus was not eradicated even though it was not demonstrated in tissue culture. It is most likely that she acquired her infection from fresh blood products, as many of these were positive on retrospective screening for CMV antibody; neither the patient nor the marrow donor had serological evidence of previous CMV infection. Her susceptibility to infection may have been further increased by the antilymphocyte globulin given post-graft.

In view of this hazard, we would advocate the exclusive use of CMV-negative donors of blood and blood products in susceptible cases, although this may be difficult, as suggested above. The similarity in clinical presentation between graft-versus-host disease and systemic CMV infection, and the possibility of their simultaneous occurrence,

emphasise the importance of screening for both conditions concurrently, especially as their respective management is quite different. The use of adenine arabinoside has produced encouraging results in this single case, but it is recommended that haematological monitoring during administration be performed daily and the drug be discontinued at the first sign of marrow suppression; the graft should ideally be well established rather than in an early emergent phase. Nevertheless, the frequency and morbidity of cytomegalovirus infection in marrow graft cases justifies stringent measures for avoidance of infection; where these measures fail, the problems of chemotherapy are not insuperable.

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## LITHIUM THERAPY IN APLASTIC ANÆMIA

SIR,—Some patients treated with lithium for psychiatric disorders develop a polymorph leucocytosis.[1-2] Since lithium stimulates human granulocyte colony growth in vitro[3] it seemed worthwhile investigating the use of lithium to stimulate granulopoiesis in patients with aplastic anæmia. We describe here the results in two patients.

A 27-year-old Libyan male developed aplastic anæmia after hepatitis. He had shown no sustained response to oxymethalone, and a course of antilymphocyte globulin in November, 1975, did not produce a complete remission. In January, 1976, his main problems were monthly blood-transfusions, occasional gingival infections requiring antibiotic treatment, and a fistula-in-ano following an ischiorectal abscess. He was receiving prednisolone 7·5 mg/day but no other systemic agents during the trial of lithium carbonate. Before the lithium therapy his blood count showed a hæmoglobin of 6 g/dl, 0–1% reticulocytes, $2{\cdot}3\times10^9$/l leucocytes, $0{\cdot}25\times10^9$/l neutrophils, and $10–80\times10^9$/l platelets. Lithium was given three times per day to achieve serum-lithium levels of 1 mmol/l. In the first 2 weeks reticulocytes rose to 3·5%, platelets to $125\times10^9$/l, and neutrophils to $0{\cdot}5–0{\cdot}8\times10^9$/l. After 4 weeks of treatment, the beneficial effects disappeared, and no further improvement was noted. The bone-marrow aspirate remained extremely hypocellular. Transfusion requirements did not alter.

A 13-year-old boy was diagnosed as having Fanconi type aplastic anæmia at the age of 6. Until 1976 he had been maintained on oxymethalone, 50 mg twice daily and did not require transfusions. In May, 1976, he had increasing anæmia, recurrent epistaxes, purpura, and mouth ulcers. His hæmoglobin was 6 g/dl, $1{\cdot}4\times10^9$/l leucocytes, $0{\cdot}3\times10^9$/l neutrophils and $10–20\times10^9$/l platelets. He was put on lithium carbonate, 750 mg/day and prednisolone 15 mg/day, which has since been progressively reduced to 7·5 mg on alternate days. The purpura stopped abruptly, the platelet-count rose to $80\times10^9$/l, reticulocytes to 3·5%, and, associated with a rise in neutrophils, the mouth ulcers healed. The rise in platelets and reticulocytes was not maintained, but the neutrophil-count rose to normal over 4 weeks. 5 months later his blood-count showed a hæmoglobin of 6 g/dl, 1–2% reticulocytes, $5\times10^9$/l leucocytes, $3\times10^9$/l neutrophils, $2\times10^9$/l lymphocytes, and $10–30\times10^9$/l platelets. There has been no serious bleeding or infection. He is at present maintained on lithium carbonate 250 mg three times a day.

Neither patient experienced any side-effects from lithium except polyuria and initial fatigue. Both patients showed similar initial hæmatological changes, but only the second patien showed a sustained rise of neutrophils to normal levels. It is possible that lithium carbonate is effective in stimulating granulopoiesis in patients with neutropenia who have sufficient reserve of granulocyte stem cells to respond. These preliminary results suggest that lithium therapy could be useful in some patients with neutropenia secondary to aplastic anæmia.

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